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(54) Title: SPLICE VARIANTS OF PREPROGLUCAGON, GLUCAGON-LIKE PEPTIDE-1 AND OXYNTOMODULIN

(57) Abstract: The present invention relates to alternative splice variants of preproglucagon, glucagon-like peptide-1 (GLP-1) and oxyntomodulin (OXM), vectors and compositions comprising same, and methods of use thereof. This invention provides peptides, nucleic acid sequences which encode same, analogs and derivatives thereof, antibodies, which specifically recognize the variant sequences, compositions comprising same and methods of use thereof.

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SPLICE VARIANTS OF PREPROGLUCAGON, GLUCAGON-LIKE PEPTIDE-1 AND OXYNTOMODULIN

FIELD OF THE INVENTION

The present invention relates to alternative splice variants of preproglucagon, glucagon-like peptide-1 (GLP-1) and oxyntomodulin (OXM), vectors and compositions comprising same, and methods of use thereof. This invention provides peptides, nucleic acid sequences which encode the same, analogs and derivatives thereof, antibodies, which specifically recognize the variant sequences, compositions comprising the same and methods of use thereof.

BACKGROUND OF THE INVENTION

Obesity and its associated disorders are common and very serious public health problems in the United States and throughout the world. Upper body obesity is the strongest risk factor known for type 2 diabetes mellitus, and is a strong risk factor for cardiovascular disease. Obesity is a recognized risk factor for hypertension, atherosclerosis, congestive heart failure, stroke, gallbladder disease, osteoarthritis, sleep apnea, reproductive disorders such as polycystic ovarian syndrome, cancers of the breast, prostate, and colon, and increased incidence of complications of general anesthesia. (see, e.g., Kopelman, Nature 404: 635-43, 2000). It reduces life-span and carries a serious risk of co-morbidities above, as well as disorders such as infections, varicose veins, acanthosis nigricans, eczema, exercise intolerance, hypercholesterolemia, cholelithiasis, orthopedic injury, and thromboembolic disease (Rissanen *et al.* BMJ 301: 835-7, 1990). Obesity is also a risk factor for the group of conditions called insulin resistance syndrome, or "Syndrome X."

Obesity is a chronic, essentially intractable metabolic disorder of ever-increasing prevalence for which no effective treatment is currently known. Therefore, clearly therapeutic treatments for obesity are very important.

Efforts to find such treatments have focused on a number of different areas, including but not limited to the use of naturally occurring hormones that have been shown to have effects in weight reduction. However, as described below, naturally

occurring hormones have a number of deficiencies, such as lack of stability in the bloodstream.

Preproglucagon, when processed, yields several proteins, which have various roles in regulating metabolism. Processing of preproglucagon occurs differently in the pancreas and in the intestine. In the pancreas, the processing leads to the formation and parallel secretion of glucagon itself, an N-terminal peptide of 30 amino acids often called glicentin-related pancreatic peptide, (GRPP), intervening peptide 1 (IP-1) and MPGF (major proglucagon derived fragment), in which the two glucagon-like sequences are buried. Glucagon seems to be the only biologically active product in the pancreas. In contrast, in the intestinal mucosa, it is glucagon that is buried in a larger molecule (oxyntomodulin), while the two glucagon-like peptides, GLP-1 and GLP-2 are formed separately.

Glucagon-like peptide 1 (GLP-1) is the most potent insulinotropic substance known, stimulating insulin secretion and potently inhibiting glucagon secretion. GLP-1 interacts directly with pancreatic β cells via its cognate receptor, thereby regulating insulin secretion via potentiation of glucose stimulated insulin release and inhibition of glucagon secretion, with the amide forms of GLP-1 (GLP-1(7-36) amide and GLP-1(7-37) amide) exhibiting the most conspicuous biological activities. Among the various biological activities of GLP-1, it exhibits pronounced blood glucose lowering effects particularly in patients with type 2 or Non-Insulin Dependent Diabetes Mellitus (NIDDM), and inhibitory effects on gastrointestinal secretion and motility, which combine to lower plasma glucose and reduce glycemic excursions. Furthermore, via its ability to enhance satiety, GLP-1 reduces food intake, thereby limiting weight gain, and may even cause weight loss. Taken together, these actions give GLP-1 a unique profile, considered highly desirable for an antidiabetic agent, particularly since the glucose dependency of its antihyperglycemic effects should minimize any risk of severe hypoglycemia (Deacon, Diabetes 53(9):2181-9, 2004).

GLP-1, however, is metabolized quickly, with a plasma half-life in humans of 2 minutes, thus the high clearance rate limits the usefulness of the native peptides. Several analogs of GLP-1 have been found to be more potent than native GLP-1, though in vivo clearance rates are still sub-optimal. Further, *in vivo* cleavage of GLP-1 and its

associated analogs by Di-Peptidyl Peptidase IV diminishes the peptide half-life, as well, which also occurs for oxyntomodulin as well.

Post-translational processing of preproglucagon in the intestine also yields the protein oxyntomodulin (OXM), which has been and is known to promote somatostatin secretion, inhibition of gastric acid secretion and promotion of insulin secretion. OXM is also thought to play a role in intestinal absorption and evacuation.

Because of their roles in regulating metabolism, variants or novel types of GLP-1 and OXM like proteins with comparable function, yet fewer limitations to their use, are necessary, and lacking at present.

SUMMARY OF INVENTION

This invention provides, in one embodiment, Glucagon-like peptide 1 (GLP-1) Splice Variants (referred to herein after as Cgen-G11), oxyntomodulin (OXM) Splice Variants (referred to herein after as Cgen-O11), Preproglucagon Splice Variants, compositions thereof and methods of using same. Methods of use include but are not limited to, treating metabolic conditions or disorders, particularly those which can be alleviated by reducing caloric availability, for example diabetes, obesity, eating disorders, insulin-resistance syndrome (Syndrome X), glucose intolerance, dyslipidemia, and cardiovascular disorders.

According to one aspect the present invention provides biologically active peptides derived from the novel Preproglucagon Splice Variant of SEQ ID NO:3. According to particular embodiments the present invention provides splice variants of GLP-1 and of OXM derived by processing of the polypeptide having the amino acid sequence set forth in SEQ ID NO:3. According to additional embodiments the present invention provides splice variants of GLP-1 and of OXM derived by processing of the corresponding polynucleotide having the sequence set forth in SEQ ID NO:4.

In one embodiment, the present invention provides an isolated Cgen-G11 GLP-1 Splice Variant polypeptide having an amino acid sequence as set forth in any one of SEQ ID NOS:7, 8, 9, 10, 11, 12, 13, 14, 58 or 59. In another embodiment, the invention provides an isolated Cgen-G11 GLP-1 Splice Variant polypeptide, wherein the polypeptide is encoded by a nucleic acid sequence as set forth in any one of SEQ ID NOS:23, 24, 25, 26, 27, 28, 29, 30, 62 or 63. In another embodiment, this invention

provides a Cgen-G11 GLP-1 Splice Variant polypeptide amidated at its C-terminus, wherein the amidated polypeptide has a sequence as set forth in any one of SEQ ID NOS:15, 16, 17, 18, 19, 20, 21, 22, 60 or 61. In another embodiment, this invention provides a Cgen-G11 GLP-1 Splice Variant polypeptide fragment comprising the C-terminus of the isolated Cgen-G11 GLP-1 Splice Variant, having an amino acid sequence as set forth in any one of SEQ ID NOS:35, 36, 37 or 38, or a fragment thereof comprising at least one Cgen-G11 epitope. In another embodiment, the present invention relates to Cgen-G11 GLP-1 Splice Variant analogs, homologs and derivatives.

According to another aspect the present invention provides antibodies capable of specifically recognizing the splice variants of the present invention from the wild type polypeptides. In one embodiment, this invention provides an antibody specifically recognizing the isolated Cgen-G11 GLP-1 Splice Variants and polypeptide fragments of this invention. Preferably such an antibody differentially recognizes Cgen-G11 GLP-1 Splice Variants of the present invention but do not recognize known GLP-1 peptides.

According to another aspect the present invention provides polynucleotides that encode the splice variants according to the present invention. According to additional aspects the invention further provides vectors, host cells, and pharmaceutical compositions comprising the nucleic acid constructs of the invention.

In particular embodiments this invention provides an isolated nucleic acid encoding for a Cgen-G11 GLP-1 Splice Variant, having a nucleotide sequence as set forth in any one of SEQ ID NOS:23, 24, 25, 26, 27, 28, 29, 30, 62 or 63, or a sequence complementary thereto. In another embodiment, this invention provides an isolated nucleic acid molecule, having a nucleotide sequence as set forth in any one of SEQ ID NOS:31, 32, 33 or 34, or a sequence complementary thereto. In another embodiment, this invention provides an oligonucleotide of at least about 12 nucleotides, wherein said oligonucleotide is specifically hybridizable with the nucleic acid molecules of this invention. In another embodiment, this invention provides compositions, cells, liposomes, and/or vectors comprising the nucleic acids of this invention.

In another embodiment, the invention provides a method for detecting GLP-1 splice variant nucleic acid sequences in a biological sample, comprising the steps of: hybridizing isolated nucleic acid molecules of this invention, or oligonucleotide fragments of at least about 12 nucleotides thereof to a nucleic acid material of the

biological sample and detecting the hybridization complex; wherein the presence of a hybridization complex correlates with the presence of a splice variant nucleic acid sequence in the biological sample.

According to one embodiment, the invention provides a method for detecting GLP-1 splice variants in a biological sample, comprising the steps of contacting the biological sample with an antibody specifically recognizing the isolated Cgen-G11 GLP-1 Splice Variant polypeptide under conditions whereby the antibody specifically interacts with a Cgen-G11 GLP-1 Splice Variant polypeptide in the biological sample but do not recognize known GLP-1 peptides, and detecting the interaction; wherein the presence of the interaction correlates with the presence of a splice variant in the biological sample. In another embodiment, this invention provides a method for treating diabetes in a subject comprising administering to the subject an amount of a Cgen-G11 GLP-1 Splice Variant having an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61 or a derivative thereof, wherein the Cgen-G11 GLP-1 Splice Variant is insulinotropic in the subject, thereby treating maturity onset diabetes mellitus in the subject.

In another embodiment, this invention provides a method for treating diabetes in a subject comprising administering to the subject an amount of an isolated nucleic acid encoding a Cgen-G11 GLP-1 Splice Variant, wherein the Cgen-G11 GLP-1 Splice Variant has an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61 or a derivative thereof, and wherein the Cgen-G11 GLP-1 Splice Variant is insulinotropic in the subject, thereby treating maturity onset diabetes mellitus in the subject.

In another embodiment, this invention provides a method for enhancing the expression of insulin in a pancreatic ß-type islet cell, comprising contacting a pancreatic ß-type islet cell with an amount of a Cgen-G11 GLP-1 Splice Variant having an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61 or a derivative thereof, under conditions that enable insulin expression, thereby enhancing the expression of insulin in a pancreatic ß-type islet cell.

In another embodiment, this invention provides a method for enhancing the expression of insulin in a pancreatic ß-type islet cell, comprising contacting a pancreatic ß-type islet cell with an amount of an isolated nucleic acid encoding a Cgen-G11 GLP-1

Splice Variant, wherein the Cgen-G11 GLP-1 Splice Variant has an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61 or a derivative thereof, under conditions that enable insulin expression, thereby enhancing the expression of insulin in a pancreatic ß-type islet cell.

In another embodiment, this invention provides a method of reducing mortality and morbidity after myocardial infraction in a subject, comprising administering to the subject an amount of a Cgen-G11 GLP-1 Splice Variant having an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61 or a derivative thereof, wherein the Cgen-G11 GLP-1 Splice Variant is at a dosage effective to normalize blood glucose, thereby reducing mortality and morbidity after myocardial infraction in the subject.

In another embodiment, this invention provides a method of reducing mortality and morbidity after myocardial infraction in a subject, comprising administering to the subject an amount of an isolated nucleic acid encoding a Cgen-G11 GLP-1 Splice Variant, wherein the Cgen-G11 GLP-1 Splice Variant has an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61 or a derivative thereof, and wherein the Cgen-G11 GLP-1 Splice Variant is at a dosage effective to normalize blood glucose, thereby reducing mortality and morbidity after myocardial infraction in the subject.

In another embodiment, this invention provides a method of attenuating post-surgical catabolic changes and insulin resistance, comprising administering to the subject an amount of a Cgen-G11 GLP-1 Splice Variant having an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61 or a derivative thereof, wherein the Cgen-G11 GLP-1 Splice Variant is insulinotropic in the subject, thereby attenuating post-surgical catabolic changes and insulin resistance.

In another embodiment, this invention provides a method of attenuating post-surgical catabolic changes and insulin resistance, comprising administering to the subject an amount of an isolated nucleic acid encoding a Cgen-G11 GLP-1 Splice Variant, wherein the Cgen-G11 GLP-1 Splice Variant has an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61 or a derivative thereof, and wherein the Cgen-G11 GLP-1 Splice Variant is insulinotropic in the subject, thereby attenuating post-surgical catabolic changes and insulin resistance.

In another embodiment, this invention provides a method of attenuating post-surgical catabolic changes and hormonal responses to stress, comprising administering

to the subject an amount of a Cgen-G11 GLP-1 Splice Variant having an amino acid sequence as set forth in any of SEQ ID NOS:7-22 or 58-61 or a derivative thereof, wherein the Cgen-G11 GLP-1 Splice Variant is insulinotropic in the subject, thereby attenuating post-surgical catabolic changes and hormonal responses to stress.

In another embodiment, this invention provides a method of attenuating post-surgical catabolic changes and hormonal responses to stress, comprising administering to the subject an amount of an isolated nucleic acid encoding a Cgen-G11 GLP-1 Splice Variant, wherein the Cgen-G11 GLP-1 Splice Variant has an amino acid sequence as set forth in any of SEQ ID NOS:7-22 or 58-61 or a derivative thereof, and wherein the Cgen-G11 GLP-1 Splice Variant is insulinotropic in the subject, thereby attenuating post-surgical catabolic changes and hormonal responses to stress.

In another embodiment, this invention provides a method of sedating a subject, comprising administering to the subject a Cgen-G11 GLP-1 Splice Variant having an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61 or a derivative thereof , in an amount sufficient to produce a sedative or anti-anxiolytic effect in the subject, thereby sedating a mammalian subject.

In another embodiment, this invention provides a method of sedating a subject, comprising administering to the subject an isolated nucleic acid encoding a Cgen-G11 GLP-1 Splice Variant, wherein the Cgen-G11 GLP-1 Splice Variant has an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61 or a derivative thereof , in an amount sufficient to produce a sedative or anti-anxiolytic effect in the subject, thereby sedating a mammalian subject.

In another embodiment, this invention provides a method of reducing body weight in a subject comprising administering to the subject a Cgen-G11 GLP-1 Splice Variant having an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61 or a derivative thereof, in an amount sufficient to cause reduction in body weight.

In another embodiment, this invention provides a method of reducing body weight in a subject comprising administering to the subject an isolated nucleic acid encoding a Cgen-G11 GLP-1 Splice Variant, wherein the Cgen-G11 GLP-1 Splice Variant has an amino acid as set forth in any one of SEQ ID NOS:7-22 or 58-61, in an amount sufficient to cause reduction in body weight.

In another embodiment, this invention provides a method of suppressing or reducing appetite in a subject, comprising administering to the subject a Cgen-G11 GLP-1 Splice Variant having an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61, or a derivative thereof, in an amount sufficient to cause suppression or reduction of appetite.

In another embodiment, this invention provides a method of suppressing or reducing appetite in a subject, comprising administering to the subject an isolated nucleic acid encoding a Cgen-G11 GLP-1 Splice Variant, wherein the Cgen-G11 GLP-1 Splice Variant has an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61 or a derivative thereof, in an amount sufficient to cause suppression or reduction of appetite.

In another embodiment, this invention provides a method of increasing an insulinotropic response in ischemia injured brain cells comprising contacting ischemia injured brain cells with a Cgen-G11 GLP-1 Splice Variant having an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61 or a derivative thereof, wherein the Cgen-G11 GLP-1 Splice Variant is insulinotropic, thereby increasing an insulinotropic response in ischemia injured brain cells.

In another embodiment, this invention provides a method of increasing an insulinotropic response in ischemia injured brain cells, comprising contacting ischemia injured brain cells with an isolated nucleic acid encoding a Cgen-G11 GLP-1 Splice Variant, wherein the Cgen-G11 GLP-1 Splice Variant has an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61 or a derivative thereof, and wherein the Cgen-G11 GLP-1 Splice Variant is insulinotropic, thereby increasing an insulinotropic response in ischemia injured brain cells.

In another embodiment, this invention provides a method of controlling stroke-related hyperglycemia in a subject, comprising administering to a subject having suffered a stroke a Cgen-G11 GLP-1 Splice Variant having an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61 or a derivative thereof, wherein the Cgen-G11 GLP-1 Splice Variant is insulinotropic and neuroprotective in the subject, thereby controlling stroke-related hyperglycemia.

In another embodiment, this invention provides a method of controlling stroke-related hyperglycemia in a subject, comprising administering to a subject having

suffered a stroke an isolated nucleic acid encoding a Cgen-G11 GLP-1 Splice Variant, wherein the Cgen-G11 GLP-1 Splice Variant has an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61 or a derivative thereof, and wherein the Cgen-G11 GLP-1 Splice Variant is insulinotropic and neuroprotective in the subject, thereby controlling stroke-related hyperglycemia.

In another embodiment, this invention provides a method of promoting weight gain in a subject, comprising administering to the subject an inhibitor of a Cgen-G11 GLP-1 Splice Variant, wherein the GLP-1 Splice Variant has an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61 or a derivative thereof, in an amount sufficient to cause weight gain in said subject.

In another embodiment, this invention provides a method of promoting weight gain in a subject, comprising administering to the subject an isolated nucleic acid capable of hybridizing to a nucleic acid encoding for a Cgen-G11 GLP-1 Splice Variant, wherein the GLP-1 Splice Variant has an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61 or a derivative thereof; and wherein the nucleic acid is administered in an amount sufficient to prevent or diminish the expression of said GLP-1 Splice Variant, thereby causing weight gain in said subject.

In yet another embodiment, the isolated nucleic acid is selected from the group consisting of: antisense nucleotide sequence, sense nucleotide sequence, short interfering RNA, ribozyme and aptamer.

In another embodiment, this invention provides an isolated Cgen-O11 OXM Splice Variant with an amino acid sequence as set forth in any one of SEQ ID NOS:41-44. In another embodiment, the invention provides a Cgen-O11 OXM Splice Variant polypeptide comprising a C-terminus of the isolated Cgen-O11 OXM Splice Variant, having an amino acid sequence as set forth in any one of SEQ ID NOS:49-52, or a fragment thereof comprising at least one Cgen-O11 epitope. In another embodiment, this invention provides antibodies specifically recognizing the Cgen-O11 OXM Splice Variants and polypeptide fragments thereof of this invention. Preferably such antibodies differentially recognize splice variants of the present invention but do not recognize known OXM peptides.

In another embodiment, this invention provides an isolated nucleic acid molecule encoding for a Cgen-O11 OXM Splice Variant, having a nucleotide sequence

as set forth in any one of SEQ ID NOS:45-48, or a sequence complementary thereto. In another embodiment, this invention provides an isolated nucleic acid molecule, having a nucleotide sequence as set forth in any one of SEQ ID NOS:53-56, or a sequence complementary thereto. In another embodiment, this invention provides an oligonucleotide of at least about 12 nucleotides, specifically hybridizable with the nucleic acid molecules of this invention. In another embodiment, this invention provides vectors, cells, liposomes and compositions comprising the isolated nucleic acids of this invention.

In another embodiment, this invention provides a method for detecting Cgen-O11 OXM Splice Variants in a biological sample, comprising the steps of: contacting a biological sample with an antibody specifically recognizing a Cgen-O11 OXM Splice Variant under conditions whereby the antibody specifically interacts with a Cgen-O11 OXM Splice Variant in the biological sample but do not recognize known OXM peptides, and detecting said interaction; wherein the presence of an interaction correlates with the presence of a splice variant in the biological sample.

In another embodiment, this invention provides a method for detecting Cgen-O11 OXM Splice Variant nucleic acid sequences in a biological sample, comprising the steps of: hybridizing the isolated nucleic acid molecules or oligonucleotide fragments of at least about 12 nucleotides thereof to a nucleic acid material of a biological sample and detecting a hybridization complex; wherein the presence of a hybridization complex correlates with the presence of a splice variant nucleic acid sequence in the biological sample.

In another embodiment, this invention provides a method for treating maturity onset diabetes mellitus in a subject comprising administering to the subject an amount of a Cgen-O11 OXM Splice Variant having an amino acid sequence as set forth in any one of SEQ ID NOS:41-44, or a derivative thereof, wherein the Cgen-O11 OXM Splice Variant is insulinotropic in the subject, thereby treating maturity onset diabetes mellitus in the subject.

In another embodiment, this invention provides a method for treating maturity onset diabetes mellitus in a subject comprising administering to the subject an amount of an isolated nucleic acid encoding a Cgen-O11 OXM Splice Variant, wherein the Cgen-O11 OXM Splice Variant has an amino acid sequence as set forth in any one of

SEQ ID NOS:41-44, or a derivative thereof, and wherein the Cgen-O11 OXM Splice Variant is insulinotropic in the subject, thereby treating maturity onset diabetes mellitus in the subject.

In another embodiment, this invention provides a method for enhancing the expression of insulin in a pancreatic ß-type islet cell, comprising contacting a pancreatic ß-type islet cell with an amount of a Cgen-O11 OXM Splice Variant having an amino acid sequence as set forth in any one of SEQ ID NOS:41-44, or a derivative thereof, under conditions that enable insulin expression, thereby enhancing the expression of insulin in a pancreatic ß-type islet cell.

In another embodiment, this invention provides a method for enhancing the expression of insulin in a pancreatic ß-type islet cell, comprising contacting a pancreatic ß-type islet cell with an amount of an isolated nucleic acid encoding a Cgen-O11 OXM Splice Variant, wherein the Cgen-O11 OXM Splice Variant has an amino acid sequence as set forth in any one of SEQ ID NOS:41-44, or a derivative thereof, under conditions that enable insulin expression, thereby enhancing the expression of insulin in a pancreatic ß-type islet cell.

In another embodiment, this invention provides a method of reducing body weight in a subject comprising administering to the subject a Cgen-O11 OXM Splice Variant having an amino acid sequence as set forth in any one of SEQ ID NOS:41-44, or a derivative thereof, in an amount sufficient to cause reduction in body weight.

In another embodiment, this invention provides a method of reducing body weight in a subject comprising administering to the subject an isolated nucleic acid encoding a Cgen-O11 OXM Splice Variant, wherein the Cgen-O11 OXM Splice Variant has an amino acid sequence as set forth in any one of SEQ ID NOS:41-44, or a derivative thereof, in an amount sufficient to cause reduction in body weight.

In another embodiment, this invention provides a method of suppressing or reducing appetite in a subject, comprising administering to the subject a Cgen-O11 OXM Splice Variant having an amino acid sequence as set forth in any one of SEQ ID NOS:41-44 or a derivative thereof, in an amount sufficient to cause suppression or reduction of appetite.

In another embodiment, this invention provides a method of suppressing or reducing appetite in a subject, comprising administering to the subject an isolated

nucleic acid encoding for a Cgen-O11 OXM Splice Variant, wherein the Cgen-O11 OXM Splice Variant has an amino acid sequence as set forth in any one of SEQ ID NOS:41-44 or a derivative thereof, in an amount sufficient to cause suppression or reduction of appetite.

In another embodiment, this invention provides a method of promoting weight gain in a subject, comprising administering to the subject an inhibitor of a Cgen-O11 OXM Splice Variant, wherein the OXM Splice Variant has an amino acid sequence as set forth in any one of SEQ ID NOS:41-44 or a derivative thereof, in an amount sufficient to cause weight gain in said subject.

In another embodiment, this invention provides a method of promoting weight gain in a subject, comprising administering to the subject an isolated nucleic acid capable of hybridizing to a nucleic acid encoding for a Cgen-O11 OXM Splice Variant, wherein the OXM Splice Variant has an amino acid sequence as set forth in any one of SEQ ID NOS:41-44 or a derivative thereof; and wherein the nucleic acid is administered in an amount sufficient to prevent or diminish the expression of said OXM Splice Variant, thereby causing weight gain in said subject.

In yet another embodiment, the isolated nucleic acid is selected from the group consisting of: antisense nucleotide sequence, sense nucleotide sequence, short interfering RNA, ribozyme and aptamer.

In another embodiment, the present invention relates to an isolated Preproglucagon Splice Variant polypeptide having an amino acid sequence as set forth in SEQ ID NO:3. In another embodiment, the invention provides an isolated Preproglucagon Splice Variant polypeptide, wherein the polypeptide is encoded by a nucleic acid sequence as set forth in SEQ ID NO:4. In another embodiment, this invention provides an antibody specifically recognizing the isolated Preproglucagon Splice Variants and polypeptide fragments of this invention. Preferably such antibodies differentially recognize splice variants of the present invention but do not recognize known preproglucagon proteins.

In another embodiment, this invention provides an isolated nucleic acid molecule encoding for a Preproglucagon Splice Variant, having a nucleotide sequence as set forth in SEQ ID NO:4, or a sequence complementary thereto. In another embodiment, this invention provides an oligonucleotide of at least about 12 nucleotides,

specifically hybridizable with the nucleic acid molecules of this invention. In another embodiment, this invention provides vectors, cells, liposomes and compositions comprising the isolated nucleic acids of this invention.

In another embodiment, this invention provides a method for detecting Preproglucagon Splice Variants in a biological sample, comprising the steps of: contacting a biological sample with an antibody specifically recognizing a Preproglucagon Splice Variant under conditions whereby the antibody specifically interacts with a Preproglucagon Splice Variant in the biological sample but do not recognize known preproglucagon proteins, and detecting said interaction; wherein the presence of an interaction correlates with the presence of a splice variant in the biological sample.

In another embodiment, this invention provides a method for detecting Preproglucagon Splice Variant nucleic acid sequences in a biological sample, comprising the steps of: hybridizing the isolated nucleic acid molecules or oligonucleotide fragments of at least about 12 nucleotides thereof to a nucleic acid material of a biological sample and detecting a hybridization complex; wherein the presence of a hybridization complex correlates with the presence of a splice variant nucleic acid sequence in the biological sample, but not with the presence of native preproglucagon.

In another embodiment, this invention provides a method for treating maturity onset diabetes mellitus in a subject comprising administering to the subject an amount of a Preproglucagon Splice Variant having an amino acid sequence as set forth in SEQ ID NO:3, or a derivative thereof, wherein the effect of the Preproglucagon Splice Variant is insulinotropic in the subject, thereby treating maturity onset diabetes mellitus in the subject.

In another embodiment, this invention provides a method for treating maturity onset diabetes mellitus in a subject comprising administering to the subject an amount of an isolated nucleic acid encoding a Preproglucagon Splice Variant, wherein the Preproglucagon Splice Variant has an amino acid sequence as set forth in SEQ ID NO:3, or a derivative thereof, and wherein the effect of said Preproglucagon Splice Variant is insulinotropic in the subject, thereby treating maturity onset diabetes mellitus in the subject.

In another embodiment, this invention provides a method for enhancing the expression of insulin in a pancreatic ß-type islet cell, comprising contacting a pancreatic ß-type islet cell with an amount of a Preproglucagon Splice Variant having an amino acid sequence as set forth in any one of SEQ ID NO:3, or a derivative thereof, under conditions that enable insulin expression, thereby enhancing the expression of insulin in a pancreatic ß-type islet cell.

In another embodiment, this invention provides a method for enhancing the expression of insulin in a pancreatic ß-type islet cell, comprising contacting a pancreatic ß-type islet cell with an amount of an isolated nucleic acid encoding a Preproglucagon Splice Variant, wherein the Preproglucagon Splice Variant has an amino acid sequence as set forth in SEQ ID NO:3, or a derivative thereof, under conditions that enable insulin expression, thereby enhancing the expression of insulin in a pancreatic ß-type islet cell.

In another embodiment, this invention provides a method of reducing body weight in a subject comprising administering to the subject a Preproglucagon Splice Variant having an amino acid sequence as set forth in SEQ ID NO:3, or a derivative thereof, in an amount sufficient to cause reduction in body weight.

In another embodiment, this invention provides a method of reducing body weight in a subject comprising administering to the subject an isolated nucleic acid encoding a Preproglucagon Splice Variant, wherein the Preproglucagon Splice Variant has an amino acid sequence as set forth in SEQ ID NO:3, or a derivative thereof, in an amount sufficient to cause reduction in body weight.

In another embodiment, this invention provides a method of suppressing or reducing appetite in a subject, comprising administering to the subject a Preproglucagon Splice Variant having an amino acid sequence as set forth in SEQ ID NO:3 or a derivative thereof, in an amount sufficient to cause suppression or reduction of appetite.

In another embodiment, this invention provides a method of suppressing or reducing appetite in a subject, comprising administering to the subject an isolated nucleic acid encoding for a Preproglucagon Splice Variant, wherein the Preproglucagon Splice Variant has an amino acid sequence as set forth in SEQ ID NO:3 or a derivative thereof, in an amount sufficient to cause suppression or reduction of appetite.

In another embodiment, this invention provides a method of promoting weight gain in a subject, comprising administering to the subject an inhibitor of a Preproglucagon Splice Variant, wherein the Preproglucagon Splice Variant has an amino acid sequence as set forth in SEQ ID NO:3 or is a derivative thereof, in an amount sufficient to cause weight gain in said subject.

In another embodiment, this invention provides a method of promoting weight gain in a subject, comprising administering to the subject an isolated nucleic acid capable of hybridizing to a nucleic acid encoding for a Preproglucagon Splice Variant, wherein the Preproglucagon Splice Variant has an amino acid sequence as set forth in SEQ ID NO:3 or is a derivative thereof; and wherein the nucleic acid is administered in an amount sufficient to prevent or diminish the expression of said Preproglucagon Splice Variant, thereby causing weight gain in said subject.

In yet another embodiment, the isolated nucleic acid is selected from the group consisting of: antisense nucleotide sequence, sense nucleotide sequence, short interfering RNA, ribozyme and aptamer.

The present invention is explained in greater detail in the description, figures, and claims below.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts an amino acid alignment for preproglucagon (SEQ ID NO:1) and a splice variant of the protein (SEQ ID NO:3). A dipeptidyl peptidase cleavage site is located between an underlined residue and the amino acid residue following it.

Figure 2 depicts the nucleic acid sequence alignment encoding for preproglucagon peptide in Figure 1 (SEQ ID NO:2), and the preproglucagon splice variant (SEQ ID NO:4).

Figure 3 schematically depicts preproglucagon processing. Pancreatic processing of preproglucagon differs in a splice variant (B) as compared to the native propeptide (A), yielding Cgen-G11 GLP-1 Splice Variants (B). Processing in the intestine of the preproglucagon splice variant (D, E) differs as compared to the native (C), yielding a Cgen-O11 OXM Splice Variants (D) or Cgen-G11 GLP-1 Splice Variants (E). Arrows indicate cleavage sites, black boxes indicate RR/KR protease sites. SP, signal peptide. IP, intervening peptide. GRPP, glucagon related pre peptide. MPGF, Major Proglucagon Derived Fragment.

Figure 4 depicts processed forms of the preproglucagon variant. The signal peptide sequence is shaded. (A) Oxyntomodulin variants. The sequence of oxyntomodulin (SEQ ID NO:41) within the preproglucagon variant is underlined. oxyntomodulin variants generated by post translational processing (SEQ ID NOS:41-44) are depicted below the preproglucagon variant. (B) GLP-1 variants. The sequence of GLP-1 (SEQ ID NO:7) within the preproglucagon variant is underlined. GLP-1 variants generated by post translational processing (SEQ ID NOS:7-14 and 58-59) are depicted below the preproglucagon variant.

Figure 5 depicts the resistance of modified GLP-1 variants to Cleavage by Dipeptidyl Peptidase IV (DPP-IV). Two N-amidated peptides were examined for their resistance to cleavage by DPP-IV: HAEGTFTSDFPRRGRHC (SEQ ID NO:19) and the modified peptide thereof: HGEGTFTSDFPRRGRHC (SEQ ID NO:64). Peptides were incubated with DPP-IV and subjected to mass spectrometry. (A) Amidated GLP-1 Splice Variant without incubation with DPP-IV; (B) Amidated GLP-1 Splice Variant after incubation with DPP-IV; (C) Modified amidated GLP-1 Splice Variant without incubation with DPP-IV; (D) Modified amidated GLP-1 Splice Variant after incubation with DPP-IV.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to novel splice variants of preproglucagon, GLP-1 and OXM, compositions thereof and methods of using same. Table 1 presents a summary of the amino acid and nucleotide sequences of the Splice Variants of this invention and the native (wild type) preproglucagon, GLP-1 and OXM:

Description	*Polypeptide SEQ ID NO:*	*Corresponding nucleotide SEQ ID NO:*
Wild type preproglucagon	1	2
Preproglucagon Splice Variant	3	4
Wild type GLP-1	5	6
GLP-1 Splice Variant 1	7	23
GLP-1 Splice Variant 2	8	24

GLP-1 Splice Variant 3	9	25
GLP-1 Splice Variant 4	10	26
GLP-1 Splice Variant 5	11	27
GLP-1 Splice Variant 6	12	28
GLP-1 Splice Variant 7	13	29
GLP-1 Splice Variant 8	14	30
GLP-1 Splice Variant 9	58	62
GLP-1 Splice Variant 10	59	63
Amidated GLP-1 Splice Variant 1	15	
Amidated GLP-1 Splice Variant 2	16	
Amidated GLP-1 Splice Variant 3	17	
Amidated GLP-1 Splice Variant 4	18	
Amidated GLP-1 Splice Variant 5	19	
Amidated GLP-1 Splice Variant 6	20	
Amidated GLP-1 Splice Variant 7	21	
Amidated GLP-1 Splice Variant 8	22	
Amidated GLP-1 Splice Variant 9	60	
Amidated GLP-1 Splice Variant 10	61	
Modified amidated GLP-1 Splice Variant 5 analog	64	
Unique C-terminus of GLP-1 Splice Variant 1	35	31
Unique C-terminus of GLP-1 Splice Variant 2	36	32
Unique C-terminus of GLP-1 Splice Variant 3	37	33
Unique C-terminus of GLP-1 Splice Variant 4	38	34
Wild type OXM	39	40

OXM Splice Variant 1	41	45
OXM Splice Variant 2	42	46
OXM Splice Variant 3	43	47
OXM Splice Variant 4	44	48
Unique C-terminus of OXM Splice Variant 1	49	53
Unique C-terminus of OXM Splice Variant 2	50	54
Unique C-terminus of OXM Splice Variant 3	51	55
Unique C-terminus of OXM Splice Variant 4	52	56

GLP-1

The hormone glucagon is known to be synthesized as a high molecular weight precursor molecule, proglucagon, whose mRNA structure and corresponding amino acid sequence are well known. Proglucagon is subsequently proteolytically cleaved into three peptides: glucagon, glucagon-like peptide 1 (GLP-1), and glucagon-like peptide 2 (GLP-2). The amino acid sequence for GLP-1 (SEQ ID NO:5), and the nucleotide sequence encoding the same (SEQ ID NO:6) have been reported (Lopez, L.C., *et al.*, Proc. Natl. Acad. Sci., USA 80:5485-5489, 1983; Bell, G. I., et al., Nature 302:716-718, 1983; Heinrich, G., et al., Endocrinol. 115:2176-2181, 1984). GLP-1 has 37 amino acids in its unprocessed form. Unprocessed GLP-1 is, however, essentially unable to mediate the induction of insulin biosynthesis. The unprocessed GLP-1 peptide is, however, naturally converted to a 31-amino acid long peptide (7-37 peptide) having amino acids 7-37 of GLP-1 ("GLP-1(7-37)"). GLP-1(7-37) can also undergo additional processing by proteolytic removal of the C-terminal glycine to produce GLP-1(7-36) which also exists predominantly with the C-terminal residue, arginine, in amidated form as argininеamide, or GLP-1 (7-36) amide. This processing occurs in the intestine and to a much lesser extent in the pancreas, and results in a polypeptide with the insulinotropic activity of GLP-1 (7-37).

In one embodiment, the term "insulinotropic" refers to an ability to stimulate, or, in another embodiment, cause the stimulation of, the synthesis or, in another

embodiment, the expression of the hormone insulin. GLP-1(7-37) and GLP-1(7-36) appear to stimulate insulin biosynthesis by pancreatic beta cells, as does, in one embodiment, the GLP-1 variant peptides of the invention.

GLP-1 Splice Variants:

In the present invention, an isolated nucleic acid was identified (SEQ ID NO:4) encoding for an alternatively spliced preproglucagon, having an amino acid sequence: MKSIYFVAGLFVMLVQGSWQRSLQDTEEKSRSFSASQADPLSDPDQMNEDKRHSQGTFT SDYSKYLDSRRAQDFVQWLMNTKRNRNNIAKRHDEFERHAEGTFTSDFPRRGRHC (SEQ ID NO:3), which upon proteolytic processing produces, in one embodiment, a Cgen-G11 GLP-1 Splice Variant (Figures 3 and 4B).

In one embodiment, "Cgen-G11" is a GLP-1 Splice Variant which is post-translationally processed from the Preproglucagon Splice Variant.

In one embodiment, the Cgen-G11 GLP-1 Splice Variant has the amino acid sequence HDEFERHAEGTFTSDFPRRGRHC (SEQ ID NO:7) or a sequence homologous thereto. In another embodiment, the Cgen-G11 GLP-1 Splice variant has the amino acid sequence HDEFERHAEGTFTSDFPRRGRH (SEQ ID NO:8), or a sequence homologous thereto. In another embodiment, the Cgen-G11 GLP-1 Splice variant has the amino acid sequence HDEFERHAEGTFTSDFPRRGR (SEQ ID NO:9), or a sequence homologous thereto. In another embodiment, the Cgen-G11 GLP-1 Splice variant has the amino acid sequence HDEFERHAEGTFTSDFPRR (SEQ ID NO:10), or a sequence homologous thereto. In another embodiment, the Cgen-G11 GLP-1 Splice variant has the amino acid sequence HDEFERHAEGTFTSDFP (SEQ ID NO:58), or a sequence homologous thereto.

In one embodiment, a Cgen-G11 GLP-1 Splice Variant having an amino acid sequence as set forth in any one of SEQ ID NOS:7-10 or 58, or a sequence homologous thereto, is insulinotropic.

In another embodiment, the Cgen-G11 GLP-1 Splice variant has the amino acid sequence HAEGTFTSDFPRRGRHC (SEQ ID NO:11), or a sequence homologous thereto. In another embodiment, the Cgen-G11 GLP-1 Splice variant has the amino acid sequence HAEGTFTSDFPRRGRH (SEQ ID NO:12), or a sequence homologous thereto. In another embodiment, the Cgen-G11 GLP-1 Splice variant has the amino acid

sequence HAEGTFTSDFPRRGR (SEQ ID NO:13), or a sequence homologous thereto. In another embodiment, the Cgen-G11 GLP-1 Splice variant has the amino acid sequence HAEGTFTSDFPRR (SEQ ID NO:14), or a sequence homologous thereto. In another embodiment, the Cgen-G11 GLP-1 Splice variant has the amino acid sequence HAEGTFTSDFP (SEQ ID NO:59), or a sequence homologous thereto.

In another embodiment, a Cgen-G11 GLP-1 Splice Variant having an amino acid sequence as set forth in any one of SEQ ID NOS:11-14 or 59 or a sequence homologous thereto, is insulinotropic. In another embodiment, the Cgen-G11 GLP-1 Splice Variant having an amino acid sequence as set forth in any one of SEQ ID NOS:11-14 or 59 is more insulinotropic than that set forth in any one of SEQ ID NOS:7-10 or 58.

Homology and homologs

The term "homology", as used herein, refers to a degree of sequence similarity in terms of shared amino acid or nucleotide sequences. There may be partial homology or complete homology (i.e., identity). For amino acid sequence homology amino acid similarity matrices (e.g. BLOSUM62, PAM70) may be utilized in different bioinformatics programs (e.g.. BLAST, FASTA, MPsrch or Scanps) and may employ the use of the Smith and Waterman algorithms, and/or global/local or BLOCKS alignments for analysis, for example. Different results may be obtained when performing a particular search with a different matrix or with a different program. Degrees of homology for nucleotide sequences are based upon identity matches with penalties made for gaps or insertions required to optimize the alignment, as is well known in the art.

Although the nucleic acid sequence of the Preproglucagon Splice Variant of this invention is 86.4% identical to that of the native preproglucagon, their amino acid sequences are only 60.6% identical (Figures 1 and 2). Moreover, both the nucleic acid sequences and the amino acid sequences of the Cgen-G11 GLP-1 Splice Variants and the Cgen-O11 OXM Splice Variants of this invention share 60% identity or less with their respective native (wild type) nucleic acid sequences and the encoded native peptides.

Therefore, as used herein, the terms "homology", "homolog" or "homologous", in any instance herein, indicate that the sequence referred to, whether an amino acid

sequence, or a nucleic acid sequence, exhibits, in one embodiment at least about 70% correspondence with the indicated sequence. In another embodiment, the amino acid sequence or nucleic acid sequence exhibits at least about 72% correspondence with the indicated sequence. In another embodiment, the amino acid sequence or nucleic acid sequence exhibits at least about 75% correspondence with the indicated sequence. In another embodiment, the amino acid sequence or nucleic acid sequence exhibits at least about 80% correspondence with the indicated sequence. In another embodiment, the amino acid sequence or nucleic acid sequence exhibits at least about 82% correspondence with the indicated sequence. In another embodiment, the amino acid sequence or nucleic acid sequence exhibits at least about 85% correspondence with the indicated sequence. In another embodiment, the amino acid sequence or nucleic acid sequence exhibits at least about 87% correspondence with the indicated sequence. In another embodiment, the amino acid sequence or nucleic acid sequence exhibits at least about 90% correspondence with the indicated sequence. In another embodiment, the amino acid sequence or nucleic acid sequence exhibits at least about 92% correspondence with the indicated sequence. In another embodiment, the amino acid sequence or nucleic acid sequence exhibits at least about 95% or more correspondence with the indicated sequence. In another embodiment, the amino acid sequence or nucleic acid sequence exhibits at least about 97% correspondence with the indicated sequence. In another embodiment, the amino acid sequence or nucleic acid sequence exhibits at least about 99% correspondence with the indicated sequence. In another embodiment, the amino acid sequence or nucleic acid sequence exhibits 95%-100% correspondence with the indicated sequence. Similarly, as used herein, the reference to a correspondence to a particular sequence includes both direct correspondence, as well as homology to that sequence as herein defined.

In one embodiment, the term "amino acid" or "amino acids" anywhere herein includes the 20 naturally occurring amino acids. In another embodiment, the term "amino acid" or "amino acids" includes those amino acids often modified post-translationally *in vivo*, such as, for example, hydroxyproline, phosphoserine and phosphothreonine. In another embodiment, particularly for analogs or homologs prepared by peptide synthesis, the term "amino acid" or "amino acids" anywhere herein includes non-coded amino acids such as, but not limited to: Abu (2-aminobutyric acid), Ahx6 (aminohexanoic acid), Ape5 (aminopentanoic acid), ArgOl (argininol), bAla (b-

Alanine), Bpa (4-Benzoylphenylalanine), Bip (Beta-[4-biphenyl]-alanine), Dab (diaminobutyric acid), Dap (Diaminopropionic acid), Dim (Dimethoxyphenylalanine), Dpr (Diaminopropionic acid), Hol (homoleucine), HPhe (Homophenylalanine), GABA (gamma aminobutyric acid), GlyNH2 (Aminoglycine), Nle (Norleucine), Nva (Norvaline), Orn (Ornithine), PheCarboxy (para carboxy Phenylalanine), PheCl (para chloro Phenylalanine), PheF (para fluoro Phenylalanine), PheMe (para methyl Phenylalanine), PheNH2 (para amino Phenylalanine), PheNO2 (para nitro Phenylalanine), Phg (Phenylglycine), Thi (Thienylalanine), 2-aminoadipic acid, hydroxylysine and isodesmosine. Certain residues may require special methods for incorporation into the peptide, and either sequential, divergent or convergent synthetic approaches to the peptide sequence are useful in this invention. In another embodiment, the term "amino acid" or "amino acids" includes both D- and L-amino acids, unless a specific configuration is indicated.

Conservative substitution of amino acids as known to those skilled in the art are within the scope of the present invention. Conservative amino acid substitutions includes replacement of one amino acid with another having the same type of functional group or side chain e.g. aliphatic, aromatic, positively charged, negatively charged. These substitutions may enhance oral bioavailability, penetration into the central nervous system, targeting to specific cell populations and the like. One of skill will recognize that individual substitutions, deletions or additions to peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. The following six groups each contain amino acids that are conservative substitutions for one another:

1) Alanine (A), Serine (S), Threonine (T);

2) Aspartic acid (D), Glutamic acid (E);

3) Asparagine (N), Glutamine (Q);

4) Arginine (R), Lysine (K);

5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and

6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

Derivatives and modifications

In another embodiment, the Splice Variants (interchangeably also referred to herein as variants) described anywhere herein, which comprise this invention, include salts and derivatives thereof. Such derived peptides include, but are not limited to, derivatives of native (human and non-human) polypeptides and their fragments. As used herein "peptide" indicates a sequence of amino acids linked by peptide bonds. The term "derived" is meant to include modified amino acid sequences and glycosylation variants, and covalent modifications of a native polypeptide. Peptides can be either linear, cyclic or branched and the like, which conformations can be achieved using methods well-known in the art.

In one embodiment, the natural aromatic amino acids, Trp, Tyr and Phe, present in any Splice Variant of this invention may be substituted for a synthetic or non-natural amino acid, such as, for example, TIC, naphthylelanine (Nol), ring-methylated derivatives of Phe, halogenated derivatives of Phe or o-methyl-Tyr.

In another embodiment, the Splice Variants of this invention may possess modifications rendering the Variants more stable while in a body or, in another embodiment, more capable of penetrating into cells. Such modifications include, but are not limited to N terminus modification, C terminus modification, peptide bond modification, including, but not limited to, CH2-NH, CH2-S, CH2-S=O, O=C-NH, CH2-O, CH2-CH2, S=C-NH, CH=CH or CF=CH, backbone modifications, and residue modification. It is to be understood that each of these modifications represents a separate embodiment of this invention.

As set forth above, the peptides of the invention can be either linear, cyclic or branched, and the like, which conformations can be achieved using methods well-known in the art. As used herein a "cyclic" peptide refers to analogs of synthetic linear peptides that can be made by chemically converting the structures to cyclic forms. Cyclization of linear peptides is accomplished either by forming a peptide bond between the free N-terminal and C-terminal ends (homodetic cyclopeptides) or by forming a new covalent bond between amino acid backbone and/or side chain groups with one another or with N- or C-terminal ends (heterodetic cyclopeptides). For example, disulfide bonds between cysteine residues may cyclize a peptide sequence. Bifunctional reagents can be used to provide a linkage between two or more amino acids of a peptide. Another approach for peptide cyclization was introduced by Gilon et

al. (Biopolymers 31:745, 1991), who proposed backbone-to-backbone cyclization of peptides. This strategy is able to effect cyclization via the carbons or nitrogens of the peptide backbone without interfering with side chains that may be crucial for interaction with the specific receptor of a given peptide. Further disclosures by Gilon and coworkers (WO 95/33765, WO 97/09344, US 5,723,575, US 5,811,392, US 5,883,293 and US 6,265,375), provided methods for producing building units required in the synthesis of backbone cyclized peptide analogs.

Homodetic cyclopeptides have no free N- or C-termini, and thus they are not susceptible to proteolysis by exopeptidases. Cyclization of linear peptides can also modulate bioactivity by increasing or decreasing the potency of binding to the target protein (Pelton, J. T., *et al.*, Proc. Natl. Acad. Sci., U.S.A., 82:236-239, 1985). Linear peptides are very flexible and tend to adopt many different conformations in solution. Cyclization acts to constrain the number of available conformations, and thus, favor the more active or inactive structures of the peptide. The immunogenicity of synthetic peptides has been correlated with the experimentally observed conformational preferences in solution (Dyson, H., *et al.*, 1988, Annual Review of Biophysics and Biophysical Chemistry, 17:305-324). Differences in immunogenicity may be indicative of differences in binding affinity of specific antibodies for cyclic peptides.

GLP-1 variants are susceptible to Dipeptidyl Peptidase IV (DPP-IV) cleavage. In another embodiment, the Cgen-G11 GLP-1 variants are rendered more resistant to protease cleavage. In one embodiment, an alanine amino acid (A) in the Cgen-G11 GLP-1 variant is replaced with another residue, rendering the Variant more resistant to protease cleavage. In one embodiment, the Cgen-G11 GLP-1 variant has an alanine residue, which is substituted with a G, S, or D-A residue, or any other natural or modified amino acid. In another embodiment, the substitution is for the alanine residue at position 8 of SEQ ID NOS:7-10 or 58 for GLP-1 splice variants. In another embodiment, the substitution is for the alanine residue at position 2 of SEQ ID NOS:11-14 or 59 for GLP-1 splice variants.

Non-limiting, illustrative examples of this latter type of substituted peptide are as follows: HAEGTFTSDFPRRGRHC wherein Ala is of the "D"-configuration; and

analogs of varying length in which G is substituted for A, including:

HGEGTFTSDFPRRGRHC;

HGEGTFTSDFPRRGRH;

HGEGTFTSDFPRRGR;

and HGEGTFTSDFPRR.

In another embodiment, the Cgen-G11 GLP-1 variants are rendered more resistant to protease cleavage through the addition of an acyl chain. In another embodiment, such a substitution delays absorption of the variant. Methods for preparing such modified Variants are well known in the art and are specified, for example, in Quantitative Drug Design, C.A. Ramsden Gd., Chapter 17.2, F. Choplin Pergamon Press, 1992.

Other methods for increasing the stability and/or activity and/or tissue distribution of peptides are also described in the art. In one embodiment, the Cgen-G11 GLP-1 Splice Variants are conjugated with a suitable stabilizing peptide sequence. Methods for preparing such modified Variants are described, for example, in WO 99/46283 and WO 98/22577. In another embodiment, the Cgen-G11 GLP-1 Splice Variants are modified through the addition of reactive groups which are capable of forming covalent bonds with one or more blood components in vivo or ex vivo. U.S. Patent No. 6,514,500 discloses a method of preparing such modified GLP-1 peptides. In another embodiment, the Cgen-G11 GLP-1 Splice Variants are linked to polyethylene glycol polymers. Several methods for pegylation of peptides are well known in the art, for example WO 04/022004 discloses a method for generating modified GLP-1 receptor agonists comprising a GLP-1 receptor agonist linked to a polyethylene glycol polymer having a molecular weight of greater than 30 kD. Other modifications include, but are not limited to: acetylation, ADP-ribosylation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, disulfide bond formation, formation of covalent cross-links, formylation, gamma-carboxylation, glycosylation, methylation, myristoylation, phosphorylation, prenylation, selenoylation and sulfation. (See, for instance Creighton, Posttranslational Covalent Modification of Proteins, W.H. Freeman and Company, New York B.C. Johnson, Ed., Academic Press, New York 1-12, 1993; Seifter, et al., Meth Enzymol 182:626-646, 1990; Rattan et al., Ann NY Acad Sci 663:48-62, 1992).

In another embodiment, the Cgen-G11 GLP-1 Splice Variant may be amidated at its C-terminus, providing a Cgen-G11 GLP-1 Splice Variant having an amino acid sequence as set forth in any one of SEQ ID NOS:15-22 or 60-61, or a sequence homologous thereto. Alternative embodiments for stabilization include C terminal reduction of the carboxy group to the corresponding alcohol, or esterifications.

It is to be understood that any Cgen-G11 GLP-1 variant-derived peptide of the present invention may be isolated, generated synthetically, obtained via translation of sequences subjected to any mutagenesis technique, as well as obtained via protein evolution techniques, well known to those skilled in the art. Cgen-G11 GLP-1 Splice Variants of this invention also include variations due to expression in various host-cell types, such as differences in the termini due to proteolytic removal of one or more terminal amino acids, and frameshifting variations, including, for example, differences in the termini due to different amino acids.

The Cgen-G11 GLP-1 Splice Variants, in one embodiment, retain GLP-1 activity. To "retain GLP-1 activity" is to have a similar level of functional activity as GLP-1. In another embodiment the Cgen-G11 GLP-1 Splice Variants possess enhanced activity, as compared to native GLP-1. In another embodiment, the Cgen-G11 GLP-1 Splice Variants exhibit enhanced stability, or in another embodiment, diminished accessibility to peptidases, as compared to native GLP-1.

Antibodies

In another embodiment, there is provided an antibody specifically recognizing a Cgen-G11 GLP-1 variant of this invention. The antibody or antibody fragment comprises an immunoglobulin specifically recognizing a Cgen-G11 GLP-1 variant or a portion thereof. The term "specifically recognizing" when referring to an antibody, refers to a binding reaction that is determinative of the presence of the protein in a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind to a particular protein at least about two times the background and do not substantially bind in a significant amount to other proteins present in the sample. Thus, preferably such an antibody differentially recognizes Cgen-G11 GLP-1 Splice Variants of the present invention but do not recognize known GLP-1 peptides.

In one embodiment, the antibody or antibody fragment specifically recognizes a protein with an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61 or a sequence homologous thereto, or a fragment thereof comprising at least one Cgen-G11 epitope. The term "epitope" refers to a site on an antigen to which B and/or T cells respond. B-cell epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. As used herein, the term "epitope" further relates to epitopes useful to distinguish between the Splice Variants of this invention and known preproglucagon-derived peptides. In another embodiment, the antibody or antibody fragment specifically recognizes an amino acid sequence as set forth in any one of SEQ ID NOS:35-38 or a sequence homologous thereto.

In one embodiment, the antibodies of this invention include intact molecules as well as functional fragments thereof, such as, for example, Fab, F(ab')2, and Fv fragments. In another embodiment, single chain antibodies ("SCA"), a genetically engineered molecule containing the variable region of the light chain and the variable region of the heavy chain, linked by a suitable polypeptide linker as a genetically fused single chain molecule as described in, for example, U.S. Patent 4,946,778, may be generated and utilized as described herein. Methods for preparing such antibodies are known in the art, and are described, for example, in Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, New York, 1988.

The present invention includes the use of serum immunoglobulins, polyclonal antibodies or fragments thereof, (i.e., an immunoreactive derivative of an antibody), or monoclonal antibodies or fragments thereof. Monoclonal antibodies or purified fragments of the monoclonal antibodies having at least a portion of an antigen-binding region, including the fragments described hereinabove, chimeric or humanized antibodies and complementarily determining regions (CDR).

Purification of these serum immunoglobulin antibodies or fragments can be accomplished by a variety of methods known to those of skill including, precipitation by ammonium sulfate or sodium sulfate followed by dialysis against saline, ion exchange chromatography, affinity or immunoaffinity chromatography as well as gel filtration, zone electrophoresis, etc. (see Goding in, Monoclonal Antibodies: Principles and Practice, 2nd ed., pp. 104-126, 1986, Orlando, Fla., Academic Press).

Methods for the generation and selection of monoclonal antibodies are well known in the art, as summarized for example in reviews such as Tramontano and Schloeder, Methods in Enzymology 178, 551-568, 1989. A recombinant Cgen-G11 GLP-1 Splice Variant of the present invention may be used to generate antibodies *in vitro* or *in vivo*.

Antibody fragments according to the present invention can be prepared by proteolytic hydrolysis of the antibody or by expression in *E. coli* or mammalian cells (e.g. Chinese hamster ovary cell culture or other protein expression systems) of DNA encoding the fragment, such as described, by Goldenberg, in U.S. Pat. Nos. 4,036,945 and 4,331,647, and Porter, R. R., Biochem. J., 73: 119-126, 1959.

The antibodies may be, in one embodiment, coupled to a detectable moiety, which may be an enzyme, a chromogen, a fluorogen, a radioactive or a light-emitting moiety. A substrate attached to a detectable moiety may be in contact with the enzyme-coupled antibody, which may therefore serve as a means of detection of a GLP-1 variant in a given sample.

Antibodies specific for Cgen-G11 GLP-1 variants may be produced by using purified GLP-1 variants for the induction of derivatized Cgen-G11 GLP-1 variant-specific antibodies. By induction of antibodies, it is intended not only the stimulation of an immune response by injection into animals, but analogous steps in the production of synthetic antibodies or other specific binding molecules such as screening of recombinant immunoglobulin libraries. Both monoclonal and polyclonal antibodies can be produced by procedures well known in the art.

Nucleic acids

The invention also provides, in another embodiment, an isolated nucleic acid molecule encoding for a Cgen-G11 GLP-1 Splice Variant. The nucleic acid sequences of this invention comprise, or are homologous to:

cacgatgaatttgagagacatgctgaagggacctttaccagtgattttcccagaagagg tcgccattgt (SEQ ID NO:23);

cacgatgaatttgagagacatgctgaagggacctttaccagtgattttcccagaagagg tcgccat (SEQ ID NO:24);

cacgatgaatttgagagacatgctgaagggacctttaccagtgattttcccagaagagg tcgc (SEQ ID NO:25);

cacgatgaatttgagagacatgctgaagggacctttaccagtgattttcccagaaga (SEQ ID NO:26);

catgctgaagggacctttaccagtgattttcccagaagaggtcgccattgt (SEQ ID NO:27);

catgctgaagggacctttaccagtgattttcccagaagaggtcgccat (SEQ ID NO:28);

catgctgaagggacctttaccagtgattttcccagaagaggtcgc (SEQ ID NO:29);

catgctgaagggacctttaccagtgattttcccagaaga (SEQ ID NO:30);

cacgatgaatttgagagacatgctgaagggacctttaccagtgattttccc (SEQ ID NO:62);

catgctgaagggacctttaccagtgattttccc (SEQ ID NO:63).

Because of the redundancy in the genetic code, it is to be understood that other nucleic acid sequences encoding for the Cgen-G11 GLP-1 Splice Variants of this invention are considered to be part of this invention, as well. Such sequences may be derived by methods well known to one in the art, including the use of computer algorithms, such as WOBBLE.

In another embodiment, the isolated nucleic acid molecule has a sequence that is complementary thereto.

In another embodiment, the invention provides an isolated nucleic acid molecule, comprising a nucleotide sequence as set forth in any one of SEQ ID NOS:31-34, or a sequence homologous or complementary thereto. These nucleic acid sequences encode the C-terminus of the Cgen-G11 GLP-1 Splice Variants, FPRRGRHC, or in another embodiment, FPRRGRH, or in another embodiment, FPRRGR, or in another embodiment, FPRR (SEQ ID NOS:35, 36, 37 or 38, respectively).

A "nucleic acid molecule" of this invention is, in one embodiment, a polymeric form of nucleotides of any length, which contain deoxyribonucleotides, ribonucleotides, and/or analogs in any combination. Nucleic acid molecules, in another embodiment, may have three-dimensional structure, and may perform, in another embodiment, any function, known or unknown. The term "nucleic acid molecule" includes, in another embodiment, double-, single-stranded, and/or triple-helical molecules. In another embodiment, any nucleic acid molecule of this invention may encompass a double stranded form, or complementary forms known, or in another embodiment, predicted to

comprise the double stranded form of DNA, or, in another embodiment, RNA or, in another embodiment, a hybrid molecule.

The following are non-limiting examples of nucleic acid molecules: a gene or gene fragment, exons, introns, mRNA, tRNA, rRNA, ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes and primers. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs, uracyl, other sugars and linking groups such as fluororibose and thiolate, and nucleotide branches. The sequence of nucleotides may be further modified after polymerization, such as by conjugation, with a labeling component. Other types of modifications included in this definition are caps, substitution of one or more of the naturally occurring nucleotides with an analog, and introduction of means for attaching the polynucleotide to proteins, metal ions, labeling components, other polynucleotides or solid support.

Nucleic acid sequence homology may be determined for any nucleic acid sequence of this invention, by, for example, the Smith-Waterman algorithm, utilized in analyzing sequence alignment protocols, as in for example, the GAP, BESTFIT, FASTA and TFASTA programs in the Wisconsin Genetics Software Package release 7.0, Genetics Computer Group, 575 Science Dr., Madison, WI).

In another embodiment, nucleic acid sequence homology may be determined for any nucleic acid sequence of this invention, by hybridization to a sequence of interest, which may be effected by stringent or moderate hybridization conditions. An example of stringent hybridization is the use of a hybridization solution containing 10% dextran sulfate, 1 M NaCl, 1% SDS and 5 x 10^6 cpm ^{32}p labeled probe, at 65 °C, with a final wash solution of 0.2 x SSC and 0.1% SDS and final wash at 65 °C; whereas an example of moderate hybridization would be the use of a hybridization solution containing 10% dextran sulfate, 1 M NaCl, 1% SDS and 5 x 10^6 cpm ^{32}p labeled probe, at 65 °C, with a final wash solution of 1 x SSC and 0.1% SDS and final wash at 50 °C.

The nucleic acids of this invention may be in either sense or antisense orientation.

The nucleic acids of the present invention can be produced by any synthetic or recombinant process such as is well known in the art. Nucleic acids according to the

invention can further be modified to alter biophysical or biological properties by means of techniques known in the art. For example, the nucleic acid can be modified to increase its stability against nucleases (e.g., "end-capping"), or to modify its lipophilicity, solubility, or binding affinity to complementary sequences.

DNA according to this invention can also be chemically synthesized by methods known in the art. For example, the DNA can be synthesized chemically from the four nucleotides in whole or in part by methods known in the art. Such methods include those described in Caruthers, Science 230(4723):281-5, 1985. DNA can also be synthesized by preparing overlapping double-stranded oligonucleotides, filling in the gaps, and ligating the ends together (see, generally, Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory Press, 1989; and Glover D M and Hames B D, eds., DNA Cloning, 2d ed., Vols. 1-4, IRL Press, Oxford, 1995). DNA expressing functional homologs of the protein can be prepared from wild-type DNA by site-directed mutagenesis (see, for example, Zoller, M. J. and Smith, M., Nucleic Acids Res. 10(20):6487-500, 1982; Zoller, M. J. and Smith, M. Methods Enzymol. 100:468-500, 1983; and Zoller, M. J. and Smith, M., DNA 3(6):479-88, 1984; McPherson ed., Directed Mutagenesis. A Practical Approach, IRL Press, Oxford, 1991. The DNA obtained can be amplified by methods known in the art. One suitable method is the polymerase chain reaction (PCR) method described in Saiki R. K. *et al.* Science 239(4839):487-91, 1988, U.S. Pat. No. 4,683,195, and Sambrook et al., 1989 cited above.

In another embodiment, this invention provides a liposome comprising the isolated nucleic acid molecules of this invention. In another embodiment, this invention provides a vector comprising the isolated nucleic acid molecules of this invention. By "vector" what is meant is a nucleic acid construct containing a sequence of interest that has been subcloned within the vector, in this case, the nucleic acid sequence encoding the Cgen-G11 GLP-1 Splice Variants. To generate the nucleic acid constructs in the context of the present invention, the polynucleotide segments encoding sequences of interest can be ligated into commercially available expression vector systems suitable for transducing/transforming mammalian cells and for directing the expression of recombinant products within the transduced/transformed cells. It will be appreciated that such commercially available vector systems can easily be modified via commonly used recombinant techniques in order to replace, duplicate or mutate existing promoter

or enhancer sequences and/or introduce any additional polynucleotide sequences such as for example, sequences encoding additional selection markers or sequences encoding reporter genes.

A vector according to the present invention may include an appropriate selectable marker. The vector may further include an origin of replication, and may be a shuttle vector, which can propagate both in bacteria, such as, for example, E. coli (wherein the vector comprises an appropriate selectable marker and origin of replication) and be compatible for propagation in vertebrate cells, or integration in the genome of an organism of choice. The vector according to this aspect of the present invention can be, for example, a plasmid, a bacmid, a phagemid, a cosmid, a phage, a virus or an artificial chromosome.

In another embodiment, there is provided a host cell comprising the isolated nucleic acid molecules and/or nucleic acid vectors as described herein. The cell may be a prokaryotic or an eukaryotic cell.

Prokaryotic cells may be used, in one embodiment, to produce the recombinant splice variants of the present invention, by methods well known in the art. In another embodiment, eukaryotic cells are used to produce the recombinant splice variants of this invention. In a further embodiment, the present invention relates to host cells containing the above-described constructs. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation (Davis, L., Dibner, M., and Battey, I. ed., Basic Methods in Molecular Biology, Elsevier Press, NY, 1986). Cell-free translation systems can also be employed to produce polypeptides using RNAs derived from the DNA constructs of the present invention.

A host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the protein include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation. Post-translational processing which cleaves a "pre-pro" form of the protein may also be important for correct insertion, folding and/or function. Different host cells such as CHO, HeLa, MDCK,

293, WI38, etc. have specific cellular machinery and characteristic mechanisms for such post-translational activities and may be chosen to ensure the correct modification and processing of the introduced, foreign protein.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express a variant product according to the present invention may be transformed using expression vectors which contain viral origins of replication or endogenous expression elements and a selectable marker gene. Following the introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clumps of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler M., *et al.*, Cell 11:223-32, 1977) and adenine phosphoribosyltransferase (Lowy I., *et al.*, Cell 22:817-23, 1980) genes which can be employed in tk- or aprt-cells, respectively. Also, antimetabolite, antibiotic or herbicide resistance can be used as the basis for selection; for example, dhfr which confers resistance to methotrexate (Wigler M., *et al.*, Proc. Natl. Acad. Sci. 77:3567-70, 1980); npt, which confers resistance to the aminoglycosides neomycin and G-418 (Colbere-Garapin, F. *et al.*, J. Mol. Biol., 150:1-14, 1981) and als or pat, which confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murry, L. E. in McGraw Hill Yearbook of Science and Technology, McGraw Hill, New York, N.Y.; pp. 191-196, 1992). Additional selectable genes have been described, for example, trpB, which allows cells to utilize indole in place of tryptophan, or hisD, which allows cells to utilize histinol in place of histidine (Hartman S. C. and R. C. Mulligan, Proc. Natl. Acad. Sci. 85:8047-51, 1988). The use of visible markers has gained popularity with such markers as anthocyanins, beta-glucuronidase and its substrate, GUS, and luciferase and its substrates, luciferin and ATP, being widely used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes, C. A. et. al., Methods Mol. Biol., 55:121-131, 1995).

Host cells transformed with a nucleotide sequence encoding a variant product according to the present invention may be cultured under conditions suitable for the expression and recovery of the encoded protein from cell culture. The product produced by a recombinant cell may be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing nucleic acid sequences encoding the variant product can be designed with signal sequences which direct secretion of the variant product through a prokaryotic or eukaryotic cell membrane.

The variant product may also be expressed as a recombinant protein with one or more additional polypeptide domains added to facilitate protein purification. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp, Seattle, Wash.). The inclusion of a protease-cleavable polypeptide linker sequence between the purification domain and the variant protein is useful to facilitate purification.

One such expression vector provides for expression of a fusion protein compromising a variant product polypeptide fused to a polyhistidine region separated by an enterokinase cleavage site. The histidine residues facilitate purification on IMIAC (immobilized metal ion affinity chromatography, as described in Porath, *et al.*, Protein Expression and Purification, 3:263-281, 1992) while the enterokinase cleavage site provides a means for isolating PSA variant polypeptide from the fusion protein. pGEX vectors (Promega, Madison, Wis.) may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to ligand-agarose beads (e.g., glutathione-agarose in the case of GST-fusions) followed by elution in the presence of free ligand.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period. Cells are typically harvested by centrifugation, disrupted by physical or

chemical means, and the resulting crude extract retained for further purification. Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, or other methods, which are well know to those skilled in the art.

The variant products can be recovered and purified from recombinant cell cultures by any of a number of methods well known in the art, including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography, and lectin chromatography. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

In another embodiment, the cells of this invention are introduced into a host. In one embodiment, such cell transfer is as a means of cell therapy.

The basis of cell therapy is to deliver a cell expressing a particular product in a tissue wherein the endogenous cell's ability to express such a product is missing or defective. Among the approaches to accomplishing cell therapy has been the use of recombinant vectors, which have been genetically engineered to carry a desired transgene, encoding for the splice variants of this invention. For example, in one embodiment, the vector is a viral vector, which infects a desired cell. In another embodiment, viral vector integrates within host cell DNA, thereby providing a continual source of expressed product. Trangene delivery may be accomplished through a variety of gene knock in methods well known in the art.

In another embodiment the vector may be introduced into desired cells by direct DNA uptake techniques, and plasmid, linear DNA or liposome mediated transduction, receptor-mediated uptake and magnetoporation methods employing calcium-phosphate mediated and DEAE-dextran mediated methods of introduction, electroporation, liposome-mediated transfection, direct injection, and receptor-mediated uptake (for further detail see, for example, Methods in Enzymology Vol. 1-317, Academic Press; Current Protocols in Molecular Biology, Ausubel F.M. *et al.* (eds.) Greene Publishing Associates, 1989; and Sambrook et al., 1989 cited above, or other standard laboratory manuals).

Such constructs can also be used in somatic and/or germ cell therapy to provide for expression of the splice variants of this invention. In one embodiment, such cells may comprise stem cells or progenitor cells. In one embodiment, such stem cells may differentiate in situ, following introduction into an appropriate host, and express the splice variants of the present invention.

In another embodiment, there is provided an oligonucleotide of at least about 12 nucleotides, specifically hybridizing with an isolated nucleic acid described herein. With respect to isolated nucleic acids encoding for a Cgen-G11 GLP-1 Splice Variant, the isolated nucleic acid, in one embodiment, have a nucleic acid sequence as set forth in any one of SEQ ID NOS:23-30 or 62-63, or a sequence homologous thereto. In another embodiment, the oligonucleotide specifically hybridizes with an isolated nucleic acid having a sequence as set forth in any one of SEQ ID NOS:31-34, or a sequence homologous thereto. In another embodiment, the oligonucleotide may hybridize with a fragment thereof. In another embodiment, the oligonucleotide is sense or antisense in orientation.

Hybridization may be conducted by any of numerous methods well known in the art, and may comprise in one embodiment, moderate conditions, or in another embodiment, under stringent conditions, or in another embodiment, under conditions therebetween. In another embodiment, this invention provides compositions comprising oligonucleotides of this invention.

In one embodiment, antisense oligonucleotides of this invention may be utilized as silencers of gene expression. Such molecules specifically bind to RNA sequences, whose expression it is desired to prevent, inhibit the translation of the RNA, thereby silencing gene expression.

In another embodiment, antisense oligonucleotides modulate gene splicing. Many genes encode pre-mRNAs containing introns that are removed by a splicing process that is directed by a complex of small nuclear ribonucleic proteins (snRNPs) called the spliceosome. Gene expression is effectively inhibited by anti-sense oligonucleotide targeting the intron/exon boundaries of splice sites because these domains direct splicing events. Antisense oligonucleotides can, in another embodiment, be designed to promote or suppress splicing at a particular site, thereby being used to enhance or limit expression of a particular Splice Variant of this invention.

Antisense oligonucleotides are typically synthesized in lengths of about 13-30 nucleotides. In one embodiment, the antisense oligonucleotides are chemically modified to prevent destruction by ubiquitous nucleases present in the body.

RNA oligonucleotides may, in another embodiment, be used for antisense inhibition as they form a stable RNA-RNA duplex with the target, suggesting efficient inhibition.

In another embodiment, synthetic oligonucleotides capable of hybridizing with double stranded DNA are utilized. According to this aspect of the invention, a triple helix is formed. Such oligonucleotides may prevent binding of transcription factors to the gene's promoter and therefore inhibit transcription. Alternatively, they may prevent duplex unwinding and, therefore, transcription of genes within the triple helical structure.

In another embodiment, ribozymes may be generated that serve to inactivate endogenous, mutated versions of native peptides from which the Splice Variants of this invention are varied, which may be a means of gene therapy, whereupon the Splice Variants are supplied in their stead.

In another embodiment, gene silencing small interfering RNAs (siRNAs) may be utilized to silence endogenous peptides from which the Splice Variants of this invention are varied, such as GLP-1, and, as will be described hereinbelow, oxyntomodulin (OXM). Duplexes consisting of between about 21-, and 23-nucleotide siRNA generated by ribonuclease III cleavage of longer dsRNAs, and by cleavage induced by other enzymes (e.g., "dicer" in D. melanogaster (Baulcombe, D. Nature 409: 295-6, 2001, and Caplen, N.J., et al. PNAS. 98: 9742-7, 2001)) thought to be similar to RNase III, or generated artificially, are the mediators of sequence specific mRNA degradation.

In another embodiment, aptamers are utilized to silence endogenous peptides from which the Splice Variants of this invention are varied, such as GLP-1, and, as will be described hereinbelow, OXM. Aptamers are specifically binding oligonucleotides for non-oligonucleotide targets that generally bind nucleic acids. The use of single-stranded DNA as an appropriate material for generating aptamers is disclosed in US Patent No. 5,840,567. Use of DNA aptamers has several advantages over RNA including increased nuclease stability, in particular plasma nuclease stability, and ease of amplification by

PCR or other methods. RNA generally is converted to DNA prior to amplification using reverse transcriptase, a process that is not equally efficient with all sequences, resulting in loss of some aptamers from a selected pool.

In another embodiment, methods of gene silencing, utilizing the reagents listed herein may serve to prevent expression of endogenous GLP-1 or, as will be described herein, comparable reagents specific for silencing of oxyntomodulin (OXM) expression. Such methods may be utilized in diseases whereby weight gain is desired, for example, such as in the treatment of anorexia, or other wasting diseases.

The antisense compounds of this invention are useful, in another embodiment, for research and diagnostics, because these compounds hybridize to nucleic acids encoding GLP-1 or OXM, enabling sandwich and other assays to easily be constructed to exploit this fact. Hybridization of the antisense oligonucleotides of the invention with a nucleic acid encoding GLP-1 or OXM, can be detected by means known in the art. Such means may include conjugation of an enzyme to the oligonucleotide, radiolabelling of the oligonucleotide or any other suitable detection means. Kits using such detection means for detecting the level of GLP-1 or OXM in a sample may also be prepared.

Oxyntomodulin:

Post-translational processing of the Preproglucagon Splice Variant of this invention, yields, in addition to GLP-1, among others, Splice Variants of Oxyntomodulin (hereinafter referred to as Cgen-O11). Native OXM has the amino acid sequence: HSQGTFTSDYSKYLDSRRAQDFVQWLMNTKRNRNNIA (SEQ ID NO:39), and a nucleic acid sequence of: cattcacagg gcacattcac cagtgactac agcaagtatc tggactccag gcgtgcccaa gattttgtgc agtggttgat gaataccaag aggaacagga ataacattgc c (SEQ ID NO:40), is produced in the intestine and hypothalamus, and is known to promote somatostatin secretion, inhibition of gastric acid secretion and promotes insulin secretion. OXM is also thought to play a role in intestinal absorption and evacuation, and to bind and activate the GLP-1 receptor, thereby initiating downstream effects of GLP-1 receptor signal transduction, thus OXM may bind receptors other than the glucagon receptor, and exert pleiotropic effects.

OXM Splice Variants:

In another embodiment, proteolytic processing of the Preproglucagon Splice Variant produces Cgen-O11 OXM Splice Variants (Figures 3 and 4A).

In one embodiment, "Cgen-O11 OXM Splice Variants" refers to peptides that are post-translationally processed from a Preproglucagon Splice Variant, as described herein.

In one embodiment, the Cgen-O11 OXM Splice Variant has the amino acid sequence:
HSQGTFTSDYSKYLDSRRAQDFVQWLMNTKRNRNNIAKRHDEFERHAEGTFTSDFPRRG RHC (SEQ ID NO:41), or a sequence homologous thereto. In another embodiment, the Cgen-O11 OXM Splice Variant has the amino acid sequence:
HSQGTFTSDYSKYLDSRRAQDFVQWLMNTKRNRNNIAKRHDEFERHAEGTFTSDFPRRG RH (SEQ ID NO:42), or a sequence homologous thereto. In another embodiment, Cgen-O11 OXM Splice Variant has the amino acid sequence:
HSQGTFTSDYSKYLDSRRAQDFVQWLMNTKRNRNNIAKRHDEFERHAEGTFTSDFPRRG R (SEQ ID NO:43), or a sequence homologous thereto. In another embodiment, the Cgen-O11 OXM Splice Variant has the amino acid sequence:
HSQGTFTSDYSKYLDSRRAQDFVQWLMNTKRNRNNIAKRHDEFERHAEGTFTSDFPRR (SEQ ID NO:44), or a sequence homologous thereto. Thus the Cgen-O11 OXM Splice Variants have a unique C terminus that comprise, or is homologous to:
KRHDEFERHAEGTFTSDFPRRGRHC, KRHDEFERHAEGTFTSDFPRRGRH, KRHDEFERHAEGTFTSDFPRRGR, or KRHDEFERHAEGTFTSDFPRR (SEQ ID NOS:49, 50, 51 and 52, respectively).

In another embodiment, this invention provides antibodies specifically recognizing Cgen-O11 OXM Splice Variants of this invention, or C-terminal fragments thereof. Preferably such antibodies differentially recognize splice variants of the present invention but do not recognize known OXM peptides. Such antibodies may comprise any of the embodiments listed herein, in terms of composition, generation, isolation and use, and are to be considered as part of this invention.

In another embodiment, the invention provides an isolated nucleic acid molecule encoding a Cgen-O11 OXM Splice Variant, comprising a nucleotide sequence as set forth in any one of SEQ ID NOS:45-48, or a sequence homologous or complementary thereto, including a nucleotide sequence for the OXM Splice Variant cat tca cag

ggc aca ttc acc agt gac tac agc aag tat ctg gac tcc agg cgt gcc caa gat ttt gtg cag tgg ttg atg aat acc aag agg aac agg aat aac att gcc aaa cgt cac gat gaa ttt gag aga cat gct gaa ggg acc ttt acc agt gat ttt ccc aga aga ggt cgc cat tgt. In another embodiment, the invention provides an isolated nucleic acid molecule encoding a C-terminal part of a Cgen-O11 OXM Splice Variant, comprising a nucleotide sequence as set forth in any one of SEQ ID NOS:53-56, or a sequence homologous or complementary thereto, including a nucleotide sequence for the OXM Splice Variant aaa cgt cac gat gaa ttt gag aga cat gct gaa ggg acc ttt acc agt gat ttt ccc aga aga ggt cgc cat tgt. In another embodiment, this invention provides vectors comprising nucleic acid sequences encoding Cgen-O11 OXM Splice Variants.

It is to be understood that a Cgen-O11 OXM Splice Variant, may comprise each and every embodiment as described herein for Cgen-G11 GLP-1 Splice Variants, applicable, for example, in terms of amino acid substitution and/or derivatization, nucleic acids encoding the Cgen-O11 OXM Splice Variant, as well as methods for their production and/or isolation.

In one embodiment, the Cgen-O11 OXM Splice Variant retains OXM activity. To "retain OXM activity" is to have a similar level of functional activity as OXM. In another embodiment the Cgen-O11 OXM Splice Variant possess enhanced activity, as compared to native OXM. In another embodiment, the Cgen-O11 OXM Splice Variant exhibit enhanced stability, or in another embodiment, diminished accessibility to peptidases, as compared to native OXM.

OXM was recently found to be a weak substrate of DPP-IV (Hinke SA *et al.*, J Biol Chem. 275(6):3827-34, 2000). Moreover, both GLUC and OXM have two cleavage sites for DPP-IV at the N-terminal end (so it can be digested twice). It is however not as common for OXM to be so cleaved as for GLP-1 and GLP-2. Furthermore, the cleavage of OXM by DPP-IV was found to be very weak in-vitro (contrary to GLP-1/2). Also, even if the extended OXM is digested by DPP-IV, the original GLP-1 N-terminus can't be cleaved by DPP-IV (as it is in the middle of the peptide).

Preproglucagon Splice Variants:

In another embodiment, the present invention provides an isolated Preproglucagon Splice Variant polypeptide having the amino acid sequence MKSIYFVAGLFVMLVQGSWQRSLQDTEEKSRSFSASQADPLSDPDQMNEDKRHSQGTFT SDYSKYLDSRRAQDFVQWLMNTKRNRNNIAKRHDEFERHAEGTFTSDFPRRGRHC (SEQ ID NO:3) or a sequence homologous thereto. In another embodiment, the invention provides an isolated Preproglucagon Splice Variant polypeptide, wherein the polypeptide is encoded by the nucleic acid sequence: atgaaaagcatttactttgtggctggattatttgtaatgctggtacaaggcagctggca acgttcccttcaagacacagaggagaaatccagatcattctcagcttcccaggcagacc cactcagtgatcctgatcagatgaacgaggacaagcgccattcacagggcacattcacc agtgactacagcaagtatctggactccaggcgtgcccaagattttgtgcagtggttgat gaataccaagaggaacaggaataacattgccaaacgtcacgatgaatttgagagacatg ctgaagggacctttaccagtgattttcccagaagaggtcgccattgttgaagaacttgg ccgcagacatgctgatggttctttctctgatgagatgaacaccattcttgataatcttg ccgccagggactttataaactggttgattcagaccaaaatcactgacaggaaataa (SEQ ID NO:4) or a sequence homologous thereto.

In another embodiment, this invention provides antibodies specifically recognizing Preproglucagon Splice Variants of this invention, or C-terminal fragments thereof. Preferably such antibodies differentially recognize splice variants of the present invention but do not recognize known preproglucagon proteins. Such antibodies may comprise any of the embodiments listed herein, in terms of composition, generation, isolation and use, and are to be considered as part of this invention.

In another embodiment, this invention provides an isolated nucleic acid molecule encoding for a Preproglucagon Splice Variant, having a nucleotide sequence as set forth in SEQ ID NO:4, or a sequence homologous or complementary thereto. In another embodiment, this invention provides an oligonucleotide of at least about 12 nucleotides, specifically hybridizable with the nucleic acid molecules of this invention. In another embodiment, this invention provides vectors, cells, liposomes and compositions comprising the isolated nucleic acids of this invention.

It is to be understood that a Preproglucagon Splice Variant, may comprise each and every embodiment as described herein for Cgen-G11 GLP-1 Splice Variants, applicable, for example, in terms of amino acid substitution and/or derivatization,

nucleic acids encoding the Preproglucagon Splice Variant, as well as methods for their production and/or isolation.

In another embodiment, upon administration to a subject, Preproglucagon Splice Variants of this invention may undergo post-translational processing, thereby generating Cgen-G11 GLP-1 Splice Variants and/or Cgen-O11 OXM Splice Variants. It is to be understood, however, that the construction and the administration route of such Preproglucagon Splice Variants would be designed to avoid any undesired effects by other proglucagon-derived peptides, such as glucagon.

Splice Variant Synthesis:

The Cgen-G11 GLP-1 and/or Cgen-O11 OXM Splice Variants of this invention, are, in one embodiment, produced as a result of post-translational processing of proglucagon/preproglucagon.

In another embodiment, the Cgen-G11 GLP-1 and/or Cgen-O11 OXM Splice Variants of this invention are produced synthetically, by any of a number of means well known in the art. The Cgen-G11 GLP-1 and/or Cgen-O11 OXM Splice Variants may, in one embodiment, be synthesized by standard methods of solid phase peptide chemistry, such as for example, via procedures described by Steward and Young (Stewart, J. M. and Young, J. D., Solid Phase Peptide Synthesis, 2nd Ed., Pierce Chemical Company, Rockford, Ill., 1984; J. M. Stewart and J. D. Young, Solid Phase Peptide Synthesis, W.H. Freeman Co. (San Francisco), 1963; and J. Meienhofer, Hormonal Proteins and Peptides, vol. 2, p. 46, Academic Press (New York), 1973). Solution phase synthetic procedures may be carried out, such as for example, as described in G. Schroder and K. Lupke, The Peptides, Vol. 1, Acacemic Press (New York). Ligation of smaller peptides, to produce the desired peptide, and other methods of peptide synthesis may be utilized, as will be known to one skilled in the art.

The Cgen-G11 GLP-1 and/or Cgen-O11 OXM Splice Variants of the invention may have, according to a preferred embodiment of this invention, the same physiological activity as the GLP-1 or OXM protein from which they are varied, respectively (although perhaps at a different level). In other embodiments, the Splice Variants of this invention may have an opposite physiological activity from the activity featured by the original peptide from which they are varied; may have a completely different, unrelated activity to the activity of the original from which they are varied; or

alternatively may have no activity at all, which may lead to various diseases or pathological conditions.

In another embodiment, the Cgen-G11 GLP-1 and/or Cgen-O11 OXM Splice Variants of this invention may differ from the original sequence in terms of their stability, clearance rate, rate of degradation, tissue and cellular distribution, ligand specification, temporal expression pattern, pattern and mechanism of up and down regulation and in other biological properties not necessarily connected to activity.

In another embodiment, the present invention provides a composition comprising isolated nucleic acid molecules encoding Cgen-G11 GLP-1 and/or Cgen-O11 OXM Splice Variants and/or Preproglucagon Splice Variants, oligonucleotides specifically hybridizing with, or vectors expressing, same. In another embodiment, the invention provides a composition comprising the Cgen-G11 GLP-1 and/or Cgen-O11 OXM Splice Variant and/or Preproglucagon Splice Variants polypeptides disclosed herein.

Compositions may include lotions, ointments, gels, creams, suppositories, drops, liquids, sprays, aerosols, powders or granules, suspensions or solutions in water or non-aqueous media, sachets, capsules or tablets. Thickeners, carriers, buffers, diluents, surface active agents, preservatives, flavorings, coloring agents, dispersing aids, emulsifiers or binders may also be included, all as well other suitable additives, all of which are well known in the art.

For example, carriers and/or diluents may include starch, mannitol, lactose, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, (or other sugars), magnesium carbonate, gelatin, oil, alcohol, detergents, emulsifiers or water (preferably sterile), each of which represents a separate embodiment of this invention. The composition may be a mixed preparation of a composition or may be a combined preparation for simultaneous, separate or sequential use (including administration). The Cgen-G11 GLP-1 and/or Cgen-O11 OXM Splice Variants and/or Preproglucagon Splice Variants may be provided as a crystalline solid, a powder, an aqueous solution, a suspension or in oil, each representing an embodiment of this invention.

The compositions may be administered in any effective, convenient manner including, for instance, administration by intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes among others. In therapy or as a

prophylactic, the active agent may be administered to an individual as an injectable composition, for example as a sterile aqueous dispersion, preferably isotonic. Compositions for oral administration may be designed to protect the active ingredient against degradation as it passes through the alimentary tract, for example, via the inclusion of a special outer coating of the formulation on a tablet or capsule, which is resistant to degradation, or allows for time release of the contents. The composition may also be packaged as a unit dose form, for example as a tablet, capsule or ampoule, for ease of administration.

A suitable administration format may best be determined by a medical practitioner for each patient individually. Various pharmaceutically acceptable carriers and their formulation are described in standard formulation treatises, e.g., Remington's Pharmaceutical Sciences by E. W. Martin, Mack Publishing Co. See also Wang, Y. J. and Hanson, M. A., Journal of Parenteral Science and Technology, Technical Report No. 10, Supp. 42:2S, 1988.

Splice variants according to the present invention can be provided as parenteral compositions for e.g., injection or infusion. Preferably, they are suspended in an aqueous carrier, for example, in an isotonic buffer solution at a pH of about 3.0 to about 8.0, preferably at a pH of about 3.5 to about 7.4, 3.5 to 6.0, or 3.5 to about 5.0. Useful buffers include sodium citrate-citric acid and sodium phosphate-phosphoric acid, and sodium acetate/acetic acid buffers. A form of repository or "depot" slow release preparation may be used so that therapeutically effective amounts of the preparation are delivered into the bloodstream over many hours or days following transdermal injection or delivery.

For use by the physician, the compositions are provided in dosage unit form containing an amount of a splice variant according to the present invention with or without another active ingredient, e.g., a food intake-reducing, plasma glucose-lowering or plasma lipid-altering agent. Therapeutically effective amounts of a splice variant according to the present invention for use in reducing nutrient availability are those that suppress appetite at a desired level. As will be recognized by one skilled in the art, an effective amount of therapeutic agent varies with many factors including the age and weight of the patient, the patient's physical condition, the blood sugar level, the weight level to be obtained, and other factors

For administration to mammals, and particularly humans, it is expected that the physician will determine the actual dosage and duration of treatment, which is most suitable for an individual and can vary with the age, weight and response of the particular individual. Dosages may also optionally be determined for GLP-1 splice variants as described herein.

The Cgen-G11 GLP-1 Splice Variants of the present invention may, in one embodiment, bind to the GLP-1 receptor. GLP-1 binding to its receptor is known to initiate a signal transduction cascade, with downstream effects on systems listed hereinbelow. In another embodiment, but without wishing to be limited by a single hypothesis, the Cgen-G11 GLP-1 Splice Variants of the present invention may bind to the same receptor as GLP-1, and/or may act at a different receptor. For example, there are alternative GLP-1 receptors (see Nishizawa M, *et al.*, J Auton Nerv Syst. 80(1-2):14-21, 2000; Ikezawa Y, *et al.*, Regul Pept. 111(1-3):207-10, 2003; and Luque MA, *et al.*, J Endocrinol. 173(3):465-73, 2002). Also, there are tissues where GLP-1 is found, but the receptor was not found. In addition, the variant may optionally bind to GLP-2 receptor as well as glucagon and oxyntomodulin (not yet identified) receptors.

In one embodiment, the Cgen-O11 OXM Splice Variants of this invention may bind to the GLP-1 receptor. OXM activates signaling pathways in cells through glucagon or GLP-1 receptors with downstream effects on systems listed hereinbelow (Schepp, W. et al, Digestion 57(6):398-405, 1996; Baggio, L. L. et al, Gastroenterology 127(2):546-58, 2004). In another embodiment, but without wishing to be limited by a single hypothesis, the Cgen-O11 OXM Splice Variants of the present invention may bind to the same receptors as OXM, and/or may act at a different receptor.

Splice Variant Protein Purification

The Cgen-G11 GLP-1 and/or Cgen-O11 OXM Splice Variants and/or Preproglucagon Splice Variants of the invention, in one embodiment, are purified by any method well known in the art. For example, the Splice Variants of the invention can be purified via column chromatography, HPLC, GLC, gel electrophoresis and immunomagnetoseparation (see for example: Strategies for Protein Purification and Characterization - A Laboratory Course Manual, CSHL Press, 1996).

Splice Variants As Competitive Inhibitors

In one embodiment, the Cgen-G11 GLP-1 and/or Cgen-O11 OXM Splice Variants are contacted with a cell in order to serve a competitive substrate for proteases that cleave the endogenous, native protein. In one embodiment, the Cgen-G11 GLP-1 Splice Variant competes for digestion by wild-type DPP-IV, thereby prolonging the circulating half-life of endogenous GLP-1. In one embodiment, the Splice Variants utilized for this aspect of the invention are engineered to be highly resistant to peptidase cleavage. In another embodiment, the Splice Variants utilized for this aspect of the invention are engineered to specifically bind to peptidases. Such methodology is well known to one skilled in the art, and may include derivatization of particular residues, such as, for example, to remove peptidase cleavage sites, wherein the Splice Variant is administered at a concentration in large excess of that of the native protein, thereby "soaking up" any available peptidase, preventing cleavage of the endogenous protein.

Splice Variants and Diabetes

The Cgen-G11 GLP-1 and/or Cgen-O11 OXM Splice Variants of the invention, in one embodiment, normalize hyperglycemia. In one embodiment, the Cgen-G11 GLP-1 and/or Cgen-O11 OXM Splice Variants normalize hyperglycemia through glucose-dependent, insulin-dependent and insulin-independent mechanisms, and, as such, are useful as primary agents for the treatment of type 2 diabetes mellitus and as adjunctive agents for the treatment of type 1 diabetes mellitus.

For example, in type 1 diabetes, any number of therapeutic regimens can be envisioned utilizing the splice variants of this invention. In one embodiment, cell therapy via implantation of pancreatic β cells engineered to express the splice variants of this invention may be accomplished, via methods well known in the art. In another embodiment, targeted delivery of vectors expressing the splice variants of this invention may be accomplished, by methods well known to one skilled in the art.

The use of an effective amount of Cgen-G11 GLP-1 and/or Cgen-O11 OXM Splice Variants as a treatment for Diabetes Mellitus are, in one embodiment, more potent than native GLP-1 and /or OXM. In another embodiment, the Cgen-G11 GLP-1 and/or Cgen-O11 OXM Splice Variants are more stable *in vivo* than native GLP-1 and/or OXM and thus are useful as a treatment for Diabetes Mellitus. In another embodiment, small amounts of the Cgen-G11 GLP-1 and/or Cgen-O11 OXM Splice Variants can be administered for effective treatment. In another embodiment, Cgen-G11

GLP-1 and/or Cgen-O11 OXM Splice Variant activity is dependent on the glucose concentration of the blood, and thus the risk of hypoglycemic side effects are greatly reduced over the risks in using current methods of treatment.

In another embodiment, there is provided a method for treating maturity onset diabetes mellitus in a subject comprising administering to the subject an amount of a GLP-1 splice variant having an amino acid sequence as set forth in any of SEQ ID NOS:7-22 or 58-61, or a sequence at least about 70% homologous thereto or a derivative thereof, wherein the Cgen-G11 GLP-1 Splice Variant is insulinotropic in the subject, thereby treating maturity onset diabetes mellitus in the subject.

In another embodiment, this invention provides a method for treating maturity onset diabetes mellitus in a subject comprising administering to the subject an amount of an isolated nucleic acid encoding a Cgen-G11 GLP-1 Splice Variant, wherein the Cgen-G11 GLP-1 Splice Variant has an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61 or a sequence at least about 70% homologous thereto or a derivative thereof, and wherein the Cgen-G11 GLP-1 Splice Variant is insulinotropic in the subject, thereby treating maturity onset diabetes mellitus in the subject.

In another embodiment, there is provided a method for treating maturity onset diabetes mellitus in a subject comprising administering to the subject an amount of a Cgen-O11 OXM Splice Variant having an amino acid sequence as set forth in any of SEQ ID NOS:41-44, or a sequence at least about 70% homologous thereto or a derivative thereof, wherein the Cgen-O11 OXM Splice Variant is insulinotropic in the subject, thereby treating maturity onset diabetes mellitus in the subject.

In another embodiment, this invention provides a method for treating maturity onset diabetes mellitus in a subject comprising administering to the subject an amount of an isolated nucleic acid encoding a Cgen-O11 OXM Splice Variant, wherein the Cgen-O11 OXM Splice Variant has an amino acid sequence as set forth in any one of SEQ ID NOS:41-44, or a sequence at least about 70% homologous thereto or a derivative thereof, and wherein the Cgen-O11 OXM Splice Variant is insulinotropic in the subject, thereby treating maturity onset diabetes mellitus in the subject.

In another embodiment, there is provided a method for treating maturity onset diabetes mellitus in a subject comprising administering to the subject an amount of a Preproglucagon Splice Variant having an amino acid sequence as set forth in SEQ ID

NO:3, or a sequence at least about 70% homologous thereto or a derivative thereof, wherein the effect of the Preproglucagon Splice Variant is insulinotropic in the subject, thereby treating maturity onset diabetes mellitus in the subject.

In another embodiment, this invention provides a method for treating maturity onset diabetes mellitus in a subject comprising administering to the subject an amount of an isolated nucleic acid encoding a Preproglucagon Splice Variant, wherein the Preproglucagon Splice Variant has an amino acid sequence as set forth in SEQ ID NO:3, or a sequence at least about 70% homologous thereto or a derivative thereof, and wherein the effect of said Preproglucagon Splice Variant is insulinotropic in the subject, thereby treating maturity onset diabetes mellitus in the subject.

In another embodiment, this invention provides a method for enhancing the expression of insulin in a pancreatic ß-type islet cell, comprising contacting a pancreatic ß-type islet cell with an amount of a Cgen-G11 GLP-1 Splice Variant having an amino acid sequence as set forth in any of SEQ ID NOS:7-22 or 58-61, or a sequence at least about 70% homologous thereto or a derivative thereof, under conditions that enable insulin expression, thereby enhancing the expression of insulin in a pancreatic ß-type islet cell.

In another embodiment, this invention provides a method for enhancing the expression of insulin in a pancreatic ß-type islet cell, comprising contacting a pancreatic ß-type islet cell with an amount of an isolated nucleic acid encoding a Cgen-G11 GLP-1 Splice Variant, wherein the Cgen-G11 GLP-1 Splice Variant has an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61 or a sequence at least about 70% homologous thereto or a derivative thereof, under conditions that enable insulin expression, thereby enhancing the expression of insulin in a pancreatic ß-type islet cell.

In another embodiment, this invention provides a method for enhancing the expression of insulin in a pancreatic ß-type islet cell, comprising contacting a pancreatic ß-type islet cell with an amount of a Cgen-O11 OXM Splice Variant having an amino acid sequence as set forth in any of SEQ ID NOS:41-44, or a sequence at least about 70% homologous thereto or a derivative thereof, under conditions that enable insulin expression, thereby enhancing the expression of insulin in a pancreatic ß-type islet cell.

In another embodiment, this invention provides a method for enhancing the expression of insulin in a pancreatic ß-type islet cell, comprising contacting a pancreatic ß-type islet cell with an amount of an isolated nucleic acid encoding a Cgen-O11 OXM Splice Variant, wherein the Cgen-O11 OXM Splice Variant has an amino acid sequence as set forth in any one of SEQ ID NOS:41-44, or a sequence at least about 70% homologous thereto or a derivative thereof, under conditions that enable insulin expression, thereby enhancing the expression of insulin in a pancreatic ß-type islet cell.

In another embodiment, this invention provides a method for enhancing the expression of insulin in a pancreatic ß-type islet cell, comprising contacting a pancreatic ß-type islet cell with an amount of a Preproglucagon Splice Variant having the amino acid sequence as set forth in SEQ ID NO:3, or a sequence at least about 70% homologous thereto or a derivative thereof, under conditions that enable insulin expression, thereby enhancing the expression of insulin in a pancreatic ß-type islet cell.

In another embodiment, this invention provides a method for enhancing the expression of insulin in a pancreatic ß-type islet cell, comprising contacting a pancreatic ß-type islet cell with an amount of an isolated nucleic acid encoding a Preproglucagon Splice Variant, wherein the Preproglucagon Splice Variant has an amino acid sequence as set forth in SEQ ID NO:3, or a sequence at least about 70% homologous thereto or a derivative thereof, under conditions that enable insulin expression, thereby enhancing the expression of insulin in a pancreatic ß-type islet cell.

In one embodiment, the term "contacting a cell", refers to any exposure of a cell to a peptide, nucleic acid, or composition of this invention. Cells may, in another embodiment, be in direct contact with compounds and compositions of the invention, or, in another embodiment, exposed indirectly, through methods well described in the art. For example, cells grown in media *in vitro*, wherein the media is supplemented with any of the GLP-1 and/or OXM Splice Variant peptides, nucleic acids, or compositions would be an example of a method of contacting a cell, considered a part of this invention.

Another example would be oral or parenteral administration of a peptide, nucleic acid or composition, whose administration results in vivo cellular exposure to these compounds, within specific sites within a body. Such administration is also considered as part of this invention, as part of what is meant by the phrase "contacting a cell".

In another embodiment, this invention provides a method for diminishing insulin resistance. Insulin resistance may be due to a decrease in binding of insulin to cell-surface receptors, or to alterations in intracellular metabolism. The first type, characterized as a decrease in insulin sensitivity, can typically be overcome by increased insulin concentration. The second type, characterized as a decrease in insulin responsiveness, cannot be overcome by large quantities of insulin. Insulin resistance following trauma can be overcome by doses of insulin that are proportional to the degree of insulin resistance, and thus is apparently caused by a decrease in insulin sensitivity.

The dose of GLP-1 and/or OXM splice variants effective to normalize a patient's blood glucose level depends on a number of factors, among which are included, without limitation, the patient's sex, weight and age, the severity of inability to regulate blood glucose, the underlying causes of inability to regulate blood glucose, whether glucose, or another carbohydrate source, is simultaneously administered, the route of administration and bioavailability, the persistence in the body, the formulation, and the potency.

For all indications, in preferred embodiments, a GLP-1 splice variant according to the present invention is preferably administered peripherally at a dose of about 1 micrograms to about 5 mg per day in single or divided doses, or at about 0.01 micrograms /kg to about 500 micrograms /kg per dose, more preferably about 0.05 micrograms /kg to about 250 micrograms /kg, most preferably below about 50 micrograms /kg. Dosages in these ranges vary with the potency of each splice variant, of course, and are readily determined by one of skill in the art.

GLP-1 Splice Variants and Nervous System Disorders

In another embodiment, the Cgen-G11 GLP-1 Splice Variants of the invention find use as a sedative. In one aspect of the invention, there is provided a method of sedating a subject, comprising administering to the subject a Cgen-G11 GLP-1 Splice Variant having an amino acid sequence as set forth in any of SEQ ID NOS:7-22 or 58-61, or a sequence at least about 70% homologous thereto or a derivative thereof, in an amount sufficient to produce a sedative or anti-anxiolytic effect in the subject, thereby sedating a mammalian subject.

In another embodiment, this invention provides a method of sedating a subject, comprising administering to the subject an isolated nucleic acid encoding a Cgen-G11 GLP-1 Splice Variant, wherein the Cgen-G11 GLP-1 Splice Variant has an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61 or a sequence at least about 70% homologous thereto or a derivative thereof, in an amount sufficient to produce a sedative or anti-anxiolytic effect in the subject, thereby sedating a mammalian subject.

In another embodiment, this invention provides a method of increasing an insulinotropic response in ischemia injured brain cells comprising contacting ischemia injured brain cells with a Cgen-G11 GLP-1 Splice Variant having an amino acid sequence as set forth in any of SEQ ID NOS:7-22 or 58-61, or a sequence at least about 70% homologous thereto or a derivative thereof, wherein the Cgen-G11 GLP-1 Splice Variant is insulinotropic, thereby increasing an insulinotropic response in ischemia injured brain cells.

In another embodiment, this invention provides a method of increasing an insulinotropic response in ischemia injured brain cells, comprising contacting ischemia injured brain cells with an isolated nucleic acid encoding a Cgen-G11 GLP-1 Splice Variant, wherein the Cgen-G11 GLP-1 Splice Variant has an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61 or a sequence at least about 70% homologous thereto or a derivative thereof, and wherein the Cgen-G11 GLP-1 Splice Variant is insulinotropic, thereby increasing an insulinotropic response in ischemia injured brain cells.

In another embodiment, this invention provides a method of controlling stroke-related hyperglycemia in a subject, comprising administering to the subject having suffered a stroke a Cgen-G11 GLP-1 Splice Variant having an amino acid sequence as set forth in any of SEQ ID NOS:7-22 or 58-61, or a sequence at least about 70% homologous thereto or a derivative thereof, wherein the Cgen-G11 GLP-1 Splice Variant is insulinotropic and neuroprotective in the subject, thereby controlling stroke-related hyperglycemia.

In another embodiment, this invention provides a method of controlling stroke-related hyperglycemia in a subject, comprising administering to a subject having suffered a stroke an isolated nucleic acid encoding a Cgen-G11 GLP-1 Splice Variant,

wherein the Cgen-G11 GLP-1 Splice Variant has an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61 or a sequence at least about 70% homologous thereto or a derivative thereof, and wherein the Cgen-G11 GLP-1 Splice Variant is insulinotropic and neuroprotective in the subject, thereby controlling stroke-related hyperglycemia.

The Cgen-G11 GLP-1 Splice Variant may be administered intracerebroventricularly, orally, subcutaneously, intramuscularly, or intravenously. Such methods are useful to treat or ameliorate nervous system conditions such as anxiety, movement disorder, aggression, psychosis, seizures, panic attacks, hysteria and sleep disorders.

GLP-1 Splice Variants and Post Surgery Treatment

The Cgen-G11 GLP-1 Splice Variants of the invention may be utilized for post surgery treatments. In another embodiment, this invention provides a method of attenuating post-surgical catabolic changes and hormonal responses to stress, comprising administering to the subject an amount of a Cgen-G11 GLP-1 Splice Variant having an amino acid sequence as set forth in any of SEQ ID NOS:7-22 or 58-61, or a sequence at least about 70% homologous thereto or a derivative thereof, wherein the Cgen-G11 GLP-1 Splice Variant is insulinotropic in the subject, thereby attenuating post-surgical catabolic changes and hormonal responses to stress.

The Cgen-G11 GLP-1 Splice Variants may be administered from about sixteen hours to about one hour before surgery begins. The length of time before surgery when the compounds used in the present invention should be administered in order to reduce catabolic effects and insulin resistance is dependent on a number of factors. These factors are generally known to the physician of ordinary skill, and include, most importantly, whether the patient is fasted or supplied with a glucose infusion or beverage, or some other form of sustenance during the preparatory period before surgery. Other important factors include the patient's sex, weight and age, the severity of any inability to regulate blood glucose, the underlying causes of any inability to regulate blood glucose, the expected severity of the trauma caused by the surgery, the route of administration and bioavailability, the persistence in the body, the formulation, and the potency of the compound administered. A preferred time interval within which to begin administration of the Cgen-G11 GLP-1 Splice Variant used in the present

invention is from about one hour to about ten hours before surgery begins. The most preferred interval to begin administration is between two hours and eight hours before surgery begins.

Insulin resistance following a particular type of surgery, elective abdominal surgery, is most profound on the first post-operative day, lasts at least five days, and may take up to three weeks to normalize Thus, the post-operative patient may be in need of administration of the Cgen-G11 GLP-1 Splice Variants used in the present invention for a period of time following the trauma of surgery that depends on factors that the physician of ordinary skill will comprehend and determine. Among these factors are whether the patient is fasted or supplied with a glucose infusion or beverage, or some other form of sustenance following surgery, and also, without limitation, the patient's sex, weight and age, the severity of any inability to regulate blood glucose, the underlying causes of any inability to regulate blood glucose, the actual severity of the trauma caused by the surgery, the route of administration and bioavailability, the persistence in the body, the formulation, and the potency of the compound administered. The preferred duration of administration of the compounds used in the present invention is not more than five days following surgery.

In another embodiment, this invention provides a method of attenuating post-surgical catabolic changes and hormonal responses to stress, comprising administering to the subject an amount of an isolated nucleic acid encoding a Cgen-G11 GLP-1 Splice Variant, wherein the Cgen-G11 GLP-1 Splice Variant has an amino acid sequence as set forth in any of SEQ ID NOS:7-22 or 58-61 or a sequence at least about 70% homologous thereto or a derivative thereof, and wherein the variant is insulinotropic in the subject, thereby attenuating post-surgical catabolic changes and hormonal responses to stress.

Splice Variants and Obesity

In another embodiment, this invention provides a method of reducing body weight in a subject comprising administering to the subject a Cgen-G11 GLP-1 Splice Variant having an amino acid sequence as set forth in any of SEQ ID NOS:7-22 or 58-61, or a sequence at least about 70% homologous thereto or a derivative thereof, in an amount sufficient to cause reduction in body weight.

In another embodiment, this invention provides a method of reducing body weight in a subject comprising administering to the subject an isolated nucleic acid encoding a Cgen-G11 GLP-1 Splice Variant, wherein the Cgen-G11 GLP-1 Splice Variant has an amino acid as set forth in any one of SEQ ID NOS:7-22 or 58-61, or a sequence at least about 70% homologous thereto or a derivative thereof, in an amount sufficient to cause reduction in body weight.

In another embodiment, this invention provides a method of reducing body weight in a subject comprising administering to the subject a Cgen-O11 OXM Splice Variant having an amino acid sequence as set forth in any of SEQ ID NOS:41-44, or a sequence at least about 70% homologous thereto or a derivative thereof, in an amount sufficient to cause reduction in body weight.

In another embodiment, this invention provides a method of reducing body weight in a subject comprising administering to the subject an isolated nucleic acid encoding a Cgen-O11 OXM Splice Variant, wherein the Cgen-O11 OXM Splice Variant has an amino acid sequence as set forth in any one of SEQ ID NOS:41-44, or a sequence at least about 70% homologous thereto or a derivative thereof, in an amount sufficient to cause reduction in body weight.

In another embodiment, the methods for body weight reduction employ combination therapy of a GLP-1 and an OXM Splice Variant.

In another embodiment, this invention provides a method of reducing body weight in a subject comprising administering to the subject a Preproglucagon Splice Variant having the amino acid sequence as set forth in SEQ ID NO:3, or a sequence at least about 70% homologous thereto or a derivative thereof, in an amount sufficient to cause reduction in body weight.

In another embodiment, this invention provides a method of reducing body weight in a subject comprising administering to the subject an isolated nucleic acid encoding a Preproglucagon Splice Variant, wherein the Preproglucagon Splice Variant has an amino acid sequence as set forth in SEQ ID NO:3, or a sequence at least about 70% homologous thereto or a derivative thereof, in an amount sufficient to cause reduction in body weight.

In another embodiment, this invention provides a method of suppressing or reducing appetite in a subject, comprising administering to a subject a Cgen-G11 GLP-1

Splice Variant and/or a Cgen-O11 OXM Splice Variant and/or a Preproglucagon Splice Variant, or derivatives thereof, in an amount sufficient to cause suppression or reduction of appetite.

In one embodiment, a Cgen-G11 GLP-1 Splice Variant and/or a Cgen-O11 OXM Splice Variant and/or a Preproglucagon Splice Variant, may be supplied in a composition suitable for oral consumption, and may be utilized as a prophylactic treatment to prevent excess weight gain. In another embodiment, administration of the Cgen-G11 GLP-1 Splice Variant and/or a Cgen-O11 OXM Splice Variant and/or a Preproglucagon Splice Variant, serves as a therapeutic for reducing excess weight. Such a reduction may be administered to clinically obese individuals, to those that are overweight, and for cosmetic weight problems. The dosage of the GLP-1 and/or OXM Splice variant and/or Preproglucagon Splice Variant are ultimately determined by the attending physician and take into consideration such factors as the Cgen-G11 GLP-1 Splice Variant and/or a Cgen-O11 OXM Splice Variant and/or Preproglucagon Splice Variant being used, animal type, age, weight, severity of symptoms and/or severity of treatment to be applied, method of administration of the medicament, adverse reaction and/or contra indications. Specific defined dosage ranges can be determined by standard designed clinical trials with patient progress and recovery being fully monitored. Additional parameters may include timing of treatment, in terms of meal intake, and adjunctive therapies including combination therapy with special diets monitoring caloric intake, in one embodiment, or in another embodiment, in conjunction with corrective surgeries. In another embodiment, such treatmetn is to accompany an exercise regimen as well.

In another embodiment, this invention provides a method of suppressing or reducing appetite in a subject, comprising administering to the subject an isolated nucleic acid encoding a Cgen-G11 GLP-1 Splice Variant, and/or a Cgen-O11 OXM Splice Variant and/or a Preproglucagon Splice Variant or a derivative thereof, in an amount sufficient to cause suppression or reduction of appetite.

GLP-1 Splice Variants and Cardiovascular Disease

In another embodiment, this invention provides a method of reducing mortality and morbidity after myocardial infraction in a subject, comprising administering to the subject an amount of a Cgen-G11 GLP-1 Splice Variant having an amino acid sequence

as set forth in any of SEQ ID NOS:7-22 or 58-61, or a sequence at least about 70% homologous thereto or a derivative thereof, wherein the Cgen-G11 GLP-1 Splice Variant is at a dosage effective to normalize blood glucose, thereby reducing mortality and morbidity after myocardial infraction in the subject.

In another embodiment, this invention provides a method of reducing mortality and morbidity after myocardial infraction in a subject, comprising administering to the subject an amount of an isolated nucleic acid encoding a Cgen-G11 GLP-1 Splice Variant, wherein the Cgen-G11 GLP-1 Splice Variant has an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61 or a sequence at least about 70% homologous thereto or a derivative thereof, and wherein the Cgen-G11 GLP-1 Splice Variant is at a dosage effective to normalize blood glucose, thereby reducing mortality and morbidity after myocardial infraction in the subject.

Monitoring the Presence of Administered Splice Variants

Cgen-G11 GLP-1 Splice Variant and/or a Cgen-O11 OXM Splice Variant activity and/or *in vivo* concentration may be measured, in one embodiment, by assaying blood drawn from a subject administered the splice variants. Blood drawn from the host at different times, enables the determination of circulating levels of the Cgen-G11 GLP-1 Splice Variant and/or Cgen-O11 OXM Splice Variant, and provides a means of assessing therapeutic dosage and administration times.

In one embodiment, the Cgen-G11 GLP-1 Splice Variant and/or Cgen-O11 OXM Splice Variant may also be monitored for their insulinotropic activity, or via HPLC-MS. In another embodiment, the Splice Variants may be monitored by ELISA or RIA. In another embodiment, the levels of a Splice Variant may be compared to that of the native protein, for diagnostic purposes, or in another embodiment, for monitoring circulating levels, or in other embodiments, for determining formulation efficacy, half-life, perfusion, and other parameters, which relate to the methods of this invention.

The insulinotropic property of the GLP-1 and/or OXM Splice Variants may be determined, in one embodiment by providing them to animal cells, or, in another embodiment, via injection into animals and monitoring the release of immunoreactive insulin (IRI) into the media or circulatory system of the animal, respectively. The presence of IRI is detected through the use of a radioimmunoassay, which can specifically detect insulin. Insulinotropic activity may also be determined, in other

embodiments, via ELISA, Western blot analysis, HPLC and other methods well known in the art.

An example of a radioimmunoassay method for insulin detection is described by Albano, J. D. M., *et al.*, (Acta Endocrinol. 70:487-509, 1972). In this assay, a phosphate/albumin buffer with a pH of 7.4 is employed. The incubation is prepared with the consecutive condition of 500 µl of phosphate buffer, 50 µl of perfusate sample or rat insulin standard in perfusate, 100 µl of anti-insulin antiserum (Wellcome Laboratories; 1:40,000 dilution), and 100 µl of [^{125}I] insulin, giving a total volume of 750 µl in a 10×75-mm disposable glass tube. After incubation for 2-3 days at 4° C., free insulin is separated from antibody-bound insulin by charcoal separation. The assay sensitivity is generally 1-2 µl U/ml. In order to measure the release of IRI into the cell culture medium of cells grown in tissue culture, one preferably incorporates radioactive label into proinsulin. Any radioactive label capable of labeling a polypeptide can be used, such as, for example, ^{3}H leucine used to obtain labeling of proinsulin. Labeling can be done for any period of time sufficient to permit the formation of a detectably labeled pool of proinsulin molecules, with cells incubated in the presence of radioactive label for, for example, a 60-minute time period. Any cell line capable of expressing insulin can be used for determining whether a Cgen-G11 GLP-1 Splice Variant has an insulinotropic effect, such as, for example, a rat insulinoma cell line, RIN-38.

The insulinotropic property of a Cgen-G11 GLP-1 Splice Variant and/or a Cgen-O11 OXM Splice Variant can also be determined by pancreatic infusion, such as via a slight modification of the method of Penhos, J. C., *et al.* (Diabetes 18:733-738, 1969). In accordance with such a method, fasted rats (preferably male Charles River strain albino rats), weighing 350-600 g, are anesthetized with an intraperitoneal injection of Amytal Sodium (Eli Lilly and Co., 160 ng/kg). Renal, adrenal, gastric, and lower colonic blood vessels are ligated. The entire intestine is resected except for about four cm of duodenum and the descending colon and rectum. Therefore, only a small part of the intestine is perfused, thus minimizing possible interference by enteric substances with insulinotropic immunoreactivity. The perfusate may be a modified Krebs-Ringer bicarbonate buffer with 4% dextran T70 and 0.2% bovine serum albumin (fraction V), and may be bubbled with 95% O_2 and 5% CO_2. A nonpulsatile flow, four-channel roller-bearing pump (Buchler polystatic, Buchler Instruments Division, Nuclear-Chicago Corp.) is preferably used, and a switch from one perfusate source to another is

preferably accomplished by switching a three-way stopcock. The manner in which perfusion is performed, modified, and analyzed, may be, for example, as described by Weir, G. C., *et al.*, (J. Clin. Investigat. 54:1403-1412, 1974).

HPLC coupled with mass spectrometry (MS) may also be utilized to assay for the presence of a Cgen-G11 GLP-1 Splice Variant and/or a Cgen-O11 OXM Splice Variant, as is well known to the skilled artisan. Two mobile phases are utilized: 0.1% TFA/water and 0.1% TFA/acetonitrile. Column temperatures can be varied as well as gradient conditions.

In another embodiment, the invention provides a method for detecting Cgen-G11 GLP-1 Splice Variant and/or Cgen-O11 OXM Splice Variant in a biological sample, comprising the steps of: contacting the biological sample with an antibody specifically recognizing a Cgen-G11 GLP-1 Splice Variant and/or a Cgen-O11 OXM Splice Variant polypeptide under conditions facilitating detection of antibody recognition of the Cgen-G11 GLP-1 Splice Variant and/or a Cgen-O11 OXM Splice Variant epitope, thereby determining the presence of the splice variant in the biological sample.

The following examples are presented in order to more fully illustrate some embodiments of the invention. They should, in no way be construed, however, as limiting the broad scope of the invention.

EXAMPLES

EXAMPLE 1

Identification of GLP-1 Splice Variants:

The method of computer algorithm identification of a Preproglucagon Splice Variant has been described in PCT application WO 01/36632, which is fully incorporated herein by reference. Briefly, mRNA sequences of the known preproglucagon gene (gi:20302161, SEQ ID NO:57) were used for screening an EST database for novel splice variants using a proprietary algorithm for clustering and assembly of nucleic acid sequences (the method for mRNA clustering and assembly used was described in US Patent Application No. 09/133,987, and the screening and annotation methods were described in US Patent Application Nos. 10/242,799; 10/426,002, all of which are incorporated herein by reference). Two EST clones were

identified from a human pancreatic islet cell library (accession numbers: D82276, SEQ ID NO:64; D82172, SEQ ID NO:65), which support the cryptic donor splice site and demonstrate novel splicing to the next exon of preproglucagon.

Two additional EST's were found to terminate exactly at the end of the splice variant exon (at the cryptic donor site) (accession numbers: BM312478, SEQ ID NO:66; BI715374, SEQ ID NO:67 from the "RH85 islet" library). All the EST and cDNA sequences identified support the existence of the novel splice variant of the preproglucagon.

A Preproglucagon Splice Variant was identified by the methods described, with the following amino acid sequence:

MKSIYFVAGLFVMLVQGSWQRSLQDTEEKSRSFSASQADPLSDPDQMNEDKRHS QGTFTSDYSKYLDSRRAQDFVQWLMNTKRNRNNIAKRHDEFERHAEGTFTSDFPRRGRH C (SEQ ID NO:3), and nucleic acid sequence,

atgaaaagcatttactttgtggctggattatttgtaatgctggtacaaggcagc tggcaacgttcccttcaagacacagaggagaaatccagatcattctcagcttcccaggc agacccactcagtgatcctgatcagatgaacgaggacaagcgccattcacagggcacat tcaccagtgactacagcaagtatctggactccaggcgtgcccaagattttgtgcagtgg ttgatgaataccaagaggaacaggaataacattgccaaacgtcacgatgaatttgagag acatgctgaagggacctttaccagtgattttcccagaagaggtcgccattgttgaagaa cttggccgcagacatgctgatggttctttctctgatgagatgaacaccattcttgataa tcttgccgccagggactttataaactggttgattcagaccaaaatcactgacaggaaat aa (SEQ ID NO:4), which differed significantly from that of the native preproglucagon (Figures 1 and 2, and SEQ ID NOS:1 and 2, respectively). From this, a Cgen-G11 GLP-1 Splice Variant was identified, with the following amino acid and nucleotide sequences: HAEGTFTSDFPRRGRHC (SEQ ID NO:11), catgctgaagggacctttaccagtgattttcccagaagaggtcgccattgt (SEQ ID NO:27), which differed from native GLP-1 (7-37), as well (SEQ ID NOS:5 and 6, respectively).

Whereas the N-terminal part of the Cgen-G11 GLP-1 Splice Variant corresponded to that of native GLP-1, the C-terminus did not, and thus the molecule as a whole shares less than 50% identity with native GLP-1.

GLP-1 is proteolytically cleaved in vivo, yielding a shorter, active protein. The Preproglucagon Splice Variant (SEQ ID NO:3) has several potential cleavage sites, generating the following Cgen-G11 GLP-1 Splice Variants in vivo: HAEGTFTSDFPRRGRHC (SEQ ID NO:11) HAEGTFTSDFPRRGRH (SEQ ID NO:12), HAEGTFTSDFPRRGR (SEQ ID NO:13) or HAEGTFTSDFPRR (SEQ ID NO:14). Each of these C-termini are unique, with respect to the native GLP-1 amino acid sequence and the nucleotide sequence encoding same (SEQ ID NOS:5 and 6, respectively), and are not found within the respective native preproglucagon sequence (SEQ ID NOS:1 and 2) as well.

Thus Cgen-G11 GLP-1 Splice Variants were identified, which shared less than 50% identity with native GLP-1.

EXAMPLE 2

Generating GLP-1 Splice Variant Polypeptides:

Polypeptides corresponding to the amino acid sequence of the Cgen-G11 GLP-1 Splice Variants are synthesized by the solid phase method as is well known in the art (Merrifield, R.. B., Chem. Soc. 85:2149, 1965; Stewart and Young, Solid Phase Peptide Synthesis, Freeman, San Francisco, 1969, pp. 27-66). It is also possible to obtain the desired polypeptides by using recombinant DNA techniques (Sambrook et al., 1989 cited above).

EXAMPLE 3

Insulinotropic GLP-1 Splice Variant Polypeptides:

The Cgen-G11 GLP-1 Splice Variants are tested in several biological systems, including conscious dog, anesthetized dog with chronic indwelling left atrial catheters, and beta TC-3 insulinoma cell line (described in D'Ambra *et al.*, Endocrinology 126:2815-2822, 1990) in cell culture. Following a bolus injection of polypeptide in a conscious dog, the insulin secretory response above basal level is determined.

EXAMPLE 4

Glucose Dependent GLP-1 Splice Variant Insulin Secretagogue Activity:

Dogs with glucose concentrations clamped at graded levels are assessed for their glucose-dependent insulinotropic response to the Cgen-G11 GLP-1 Splice Variants.

Varying dosages of the peptides are administered, and dosages which do not stimulate insulin release at fasting glucose concentrations of 50-75 mg/dL (such as 0.1 nmol peptides, given as a bolus) yet are able to produce a peak insulin response of one-fold above basal when given to dogs in a clamped, hyperglycemic state are determined.

The peptides may also be compared in order to determine which provides a greater insulin secretory response.

EXAMPLE 5

GLP-1 Splice Variants Direct Activity on Pancreatic Beta Cells:

Beta TC-3 cells are cultured in serum-containing media in 48-well culture dishes to confluency. Cells are tested in Earle's balanced salt solution containing IBMX, BSA and 16.7 mM glucose with graded concentrations of the Cgen-G11 GLP-1 Splice Variants for 1 hour at 37 °C prior to supernatant collection and assay for insulin concentration.

EXAMPLE 6

GLP-1 Splice Variants Reduction of Hyperglycemia in a Diabetic Animal Model:

The db/db mouse is a genetically obese and diabetic strain of mouse. The db/db mouse develops hyperglycemia and hyperinsulinemia concomitant with its development of obesity and thus serves as a model of obese type 2 diabetes (NIDDM). Sub-orbital sinus blood samples are drawn from 11-week old db/db mice purchased prior to and 60 minutes post-intraperitoneal injection of the Cgen-G11 GLP-1 Splice Variants. Blood glucose measurements are conducted with the aid of a glucose meter and reduction of blood glucose levels in the diabetic animals is assessed.

EXAMPLE 7

Generating OXM Splice Variant Polypeptides:

Polypeptides corresponding to the amino acid sequence of the Cgen-O11 OXM Splice Variants are synthesized by the solid phase method as is well known in the art (Merrifield, R.B., Chem. Soc. 85:2149 1965; Stewart and Young, Solid Phase Peptide Synthesis, Freeman, San Francisco, 1969, pp. 27-66). It is also possible to obtain the desired polypeptides by using recombinant DNA techniques (Sambrook et al., 1989 cited above)

EXAMPLE 8

Insulinotropic OXM Splice Variant Polypeptides:

The OXM Splice Variants are tested in several biological systems, including conscious dog, anesthetized dog with chronic indwelling left atrial catheters, and beta TC-3 insulinoma cell line (described in D'Ambra et al., Endocrinology 126:2815-2822, 1990) in cell culture. Following a bolus injection of polypeptide in a conscious dog, the insulin secretory response above basal level is determined.

EXAMPLE 9

Glucose Dependent OXM Splice Variant Insulin Secretagogue Activity:

Dogs with glucose concentrations clamped at graded levels are assessed for their glucose-dependent insulinotropic response to the Cgen-O11 OXM Splice Variants. Varying dosages of the peptides are administered, and dosages which do not stimulate insulin release at fasting glucose concentrations of 50-75 mg/dL (such as 0.1 nmol peptides, given as a bolus) yet are able to produce a peak insulin response of one-fold above basal when given to dogs in a clamped, hyperglycemic state are determined. The peptides may also be compared in order to determine which provides a greater insulin secretory response.

EXAMPLE 10

OXM Splice Variants Direct Activity on Pancreatic Beta Cells:

Beta TC-3 cells are cultured in serum-containing media in 48-well culture dishes to confluency. Cells are tested in Earle's balanced salt solution containing IBMX, BSA

and 16.7 mM glucose with graded concentrations of the Cgen-O11 OXM Splice Variants for 1 hour at 37 °C prior to supernatant collection and assay for insulin concentration.

EXAMPLE 11

OXM Splice Variants reduction of hyperglycemia in a diabetic animal model:

The db/db mouse is a genetically obese and diabetic strain of mouse. The db/db mouse develops hyperglycemia and hyperinsulinemia concomitant with its development of obesity and thus serves as a model of obese type 2 diabetes (NIDDM). Sub-orbital sinus blood samples are drawn from 11-week old db/db mice purchased prior to and 60 minutes post-intraperitoneal injection of the Cgen-O11 OXM Splice Variants. Blood glucose measurements are conducted with the aid of a glucose meter and reduction of blood glucose levels in the diabetic animals is assessed.

EXAMPLE 12

OXM and GLP-1 variant Reduction of Body Weight in Animal Models:

Cumulative food intake in grams is measured over the course of 24 hours following intravenous or intraperitoneal injection of Cgen-G11 GLP-1 Splice Variants and/or Cgen-O11 OXM variants, in fasted rats and/or sated rats. Dose-dependent decreases in food intake are determined in treated rats versus placebo treated controls, as well as single versus multiple injections. The effect of the time of day of feeding and/or administration of the Splice Variants is determined as well. At the conclusion of the experiment, in addition to the measurement of food intake, as well as overall body weight, gastric emptying is determined in the rats, as well, with the contents in dry weight expressed as a percentage of food intake during the feeding period. Decreases in fasting-induced refeeding following injection of Cgen-G11 GLP-1 and/or Cgen-O11 OXM Splice Variants are measured, as well.

Adult rats can also be cannulated and infused with the Splice Variants and placebo controls, with delivery following a 24 hour fast, or measured in non-fasted animals, and food intake is measured at multiple time points following delivery of the Splice Variants.

EXAMPLE 13

Resistance of Modified GLP-1 variant to Cleavage:

GLP-1 is known to be readily and rapidly cleaved by Dipeptidyl Peptidase IV (DPP-IV/CD26) in serum, which is a clear drawback for this known peptide as a therapeutic peptide.

The GLP1 variants are believed to be resistant to such cleavage in serum by DPP-IV, as previously described. Additional modifications of a GLP-1 variant according to the present invention were made, to further increase the potential resistance of the peptide to cleavage.

DPP-IV cleaves GLP-1 at the alanine residue at position 2, which not only inactivates GLP-1 but (it is hypothesized) could cause the cleaved protein to act as an antagonist at the GLP-1 receptor. Modifications of a GLP-1 variant according to the present invention were checked to see if this problem could be at least ameliorated.

Two peptides were examined for their resistance to cleavage by DPP-IV: HAEGTFTSDFPRRGRHC (MW = 1973 Da) and the modified peptide thereof: HGEGTFTSDFPRRGRHC (MW = 1959 Da). Both peptides were N-terminal amidated (SEQ ID NOS:19 and 64, respectively).

The following method was used to test cleavage. 10 ng of DPP-IV was incubated with 5 nmol peptide in 25 mM tris pH 8.0 4h at RT (room temperature). Reaction was stopped by freezing the samples, and the resulting samples were subjected to mass spectrometry.

Samples for mass spectroscopy were prepared as follows. Aliquots of the resulting peptide mixtures were dissolved in 0.1% TFA and used for mass spectroscopy using the fast evaporation method. Matrix surfaces were made from α-cyano-4-hydroxycinnamic acid (4-HCCA) by the fast evaporation method (Vorm, O. et al., Anal. Chem. 66, 3281-3287, 1994; Jensen, O.N. et al., Rapid Communications in Mass Spectrometry 10, 1371-1378, 1996). Two parts of HCCA and one part of NC (membrane from Bio-Rad) were dissolved in acetone-propan-2-ol (4:1) to final concentrations of 20 mg (HCCA) and 10 mg ml-1 (NC). A 0.5 ml volume of this solution was deposited on the target and allowed to spread and dry. 0.5 ml of sample

solution in 0.1% TFA were deposited on the matrix layer. After solvent evaporation the samples were washed 1-3 times with 0.1% TFA.

Molecular mass measurements were performed on a Bruker Reflex III™ matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) mass spectrometer (Bruker, Bremen, Germany) equipped with delayed ion extraction, reflector and a 337 nm nitrogen laser. Each mass spectrum was generated from accumulated data of 200 laser shots.

The following conditions were followed:

a) DPP-IV in buffer

b) GLP-1 variant +/- DPP-IV

c) GLP-1 modified variant +/- DPP-IV

Results were found as shown in Figure 5. Briefly, Figures 5A and 5B relate to the GLP-1 variant, while Figures 5C and 5D relate to the modified GLP-1 variant. Figure 5A shows the MALDI-TOF MS (mass spectroscopy) of the GLP-1 variant without incubation with DPP-IV, while Figure 5B shows the MALDI-TOF MS of the GLP-1 variant after incubation with DPP-IV. Clearly, there is a difference in the peaks for Figures 5A and 5B, with the peak at 1973 Da disappearing and two new peaks appearing in Figure 5B at 1813 and 1764 Da. However, no such difference between peaks is seen between Figures 5C and 5D, as peak 1959 Da is present in both, showing that the modified GLP-1 variant is clearly resistant to cleavage by DPP-IV and hence is expected to be more stable in the blood and/or body of the subject.

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying current knowledge, readily modify and/or adapt for various applications such specific embodiments without undue experimentation and without departing from the generic concept, and, therefore, such adaptations and modifications should and are intended to be comprehended within the meaning and range of equivalents of the disclosed embodiments. Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives,

modifications and variations that fall within the spirit and broad scope of the appended claims.

It is to be understood that the phraseology or terminology employed herein is for the purpose of description and not of limitation. The means, materials, and steps for carrying out various disclosed chemical structures and functions may take a variety of alternative forms without departing from the invention.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub combination.

All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention.

CLAIMS

What is claimed is:

1. A biologically active peptide derived from a preproglucagon splice variant having the amino acid sequence set forth in SEQ ID NO:3.

2. The biologically active peptide of claim 1 wherein the peptide is a splice variant selected from glucagon like protein –1 and oxyntomodulin.

3. An isolated Cgen-G11 GLP-1 splice variant having an amino acid sequence as set forth in any one of SEQ ID NOS:7-14 or 58-59.

4. The isolated Cgen-G11 GLP-1 splice variant polypeptide of claim 3, wherein said polypeptide is amidated at its C-terminus.

5. The isolated Cgen-G11 GLP-1 splice variant polypeptide of claim 4, wherein said amidated polypeptide has a sequence as set forth in any one of SEQ ID NOS:15-22 or 60-61.

6. An isolated Cgen-G11 GLP-1 splice variant peptide fragment comprising the C-terminus of the isolated Cgen-G11 GLP-1 splice variant of claim 3, having an amino acid sequence as set forth in any one of SEQ ID NOS:35-38, or a fragment thereof comprising at least one Cgen-G11 epitope.

7. An antibody specifically recognizing an epitope of the isolated Cgen-G11 GLP-1 splice variant peptide fragment of claim 6.

8. An antibody specifically recognizing the isolated Cgen-G11 GLP-1 splice variant polypeptide of claim 3.

9. The antibody of claim 7 or 8, wherein said antibody is coupled to a detectable moiety.

10. The antibody of claim 9, wherein said detectable moiety is an enzyme, a chromogen, a fluorogen, a radioactive or a light-emitting moiety.

11. A pharmaceutical composition comprising the isolated Cgen-G11 GLP-1 splice variant polypeptide of claim 3, further comprising a pharmaceutically acceptable carrier or excipient.

12. An isolated polynucleotide encoding a Cgen-G11 GLP-1 splice variant according to claim 3.

13. The isolated nucleic acid molecule encoding for a Cgen-G11 GLP-1 splice variant according to claim 12, having a nucleotide sequence as set forth in any one of SEQ ID NOS:23-30 or 62-63, or a sequence complementary thereto.

14. A vector comprising the isolated nucleic acid molecule of claim 13.

15. A composition, liposome or cell comprising the vector of claim 14.

16. A composition, liposome or cell comprising the isolated nucleic acid molecule of claim 13.

17. An isolated nucleic acid molecule, having a nucleotide sequence as set forth in any one of SEQ ID NOS:31-34, or a sequence complementary thereto.

18. A vector comprising the isolated nucleic acid molecule of claim 17.

19. An oligonucleotide of at least about 12 nucleotides specifically hybridizable with the nucleic acid molecule of claim 17.

20. An isolated Cgen-O11 OXM splice variant having an amino acid sequence as set forth in any one of SEQ ID NOS:41-44.

21. A Cgen-O11 OXM splice variant peptide fragment comprising the C-terminus of the isolated Cgen-O11 OXM Splice Variant of claim 20, having an amino acid sequence as set forth in any one of SEQ ID NOS:49-52, or a fragment thereof comprising at least one Cgen-O11 epitope.

22. An antibody specifically recognizing an epitope of the isolated Cgen-O11 OXM splice variant peptide fragment of claim 21.

23. An antibody specifically recognizing the isolated Cgen-O11 OXM splice variant of claim 20.

24. The antibody of claim 22 or 23, wherein said antibody is coupled to a detectable moiety.

25. The antibody of claim 24, wherein said detectable moiety is an enzyme, a chromogen, a fluorogen, a radioactive or a light-emitting moiety.

26. A pharmaceutical composition comprising the isolated Cgen-O11 OXM splice variant of claim 20, further comprising a pharmaceutically acceptable carrier or excipient.

27. An isolated polynucleotide encoding a Cgen-O11 OXM splice variant according to claim 20.

28. The isolated nucleic acid molecule encoding a Cgen-O11 OXM splice variant according to claim 27, having a nucleotide sequence as set forth in any one of SEQ ID NOS:45-48, or a sequence complementary thereto.

29. A vector comprising the isolated nucleic acid molecule of claim 28.

30. A composition, liposome or cell comprising the vector of claim 29.

31. A composition, liposome or cell comprising the isolated nucleic acid molecule of claim 28.

32. An isolated nucleic acid molecule, having a nucleotide sequence as set forth in any one of SEQ ID NOS:53-56, or a sequence complementary thereto.

33. A vector comprising the isolated nucleic acid molecule of claim 32.

34. An oligonucleotide of at least about 12 nucleotides, specifically hybridizable with the nucleic acid molecule of claim 32.

35. A method for detecting GLP-1 splice variants in a biological sample, comprising the steps of: contacting said biological sample with an antibody specifically recognizing the isolated Cgen-G11 GLP-1 splice variant polypeptide of claim 3 under conditions whereby said antibody specifically interacts with a Cgen-G11 GLP-1 splice variant polypeptide in said biological sample, and detecting said

interaction; wherein the presence of said interaction correlates with the presence of a splice variant in the biological sample.

36. The method of claim 35, wherein said antibody specifically recognizes a Cgen-G11 GLP-1 splice variant peptide fragment of claim 6.

37. A method for detecting GLP-1 splice variant nucleic acid sequences in a biological sample, comprising the steps of: hybridizing the isolated nucleic acid molecule of claim 13, or an oligonucleotide fragment of at least about 12 nucleotides thereof to a nucleic acid material of said biological sample and detecting said hybridization complex; wherein the presence of said hybridization complex correlates with the presence of a splice variant nucleic acid sequence in the said biological sample.

38. A method for detecting GLP-1 splice variant nucleic acid sequences in a biological sample, comprising the steps of: hybridizing an isolated nucleic acid molecule encoding for a Cgen-G11 GLP-1 splice variant polypeptide fragment of claim 6, or a fragment of at least about 12 nucleotides thereof to a nucleic acid material of said biological sample and detecting said hybridization complex; wherein the presence of said hybridization complex correlates with the presence of a splice variant nucleic acid sequence in the said biological sample.

39. A method for treating maturity onset diabetes mellitus in a subject comprising administering to the subject an amount of a Cgen-G11 GLP-1 splice variant having an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61, or a derivative thereof, wherein the Cgen-G11 GLP-1 splice variant is insulinotropic in said subject, thereby treating maturity onset diabetes mellitus in said subject.

40. A method for treating maturity onset diabetes mellitus in a subject comprising administering to the subject an amount of an isolated nucleic acid encoding for a Cgen-G11 GLP-1 splice variant, wherein the Cgen-G11 GLP-1 splice variant has an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61, or a derivative thereof, and said Cgen-G11 GLP-1 splice variant is insulinotropic in said subject, thereby treating maturity onset diabetes mellitus in said subject.

41. The method of claim 40, wherein said isolated nucleic acid is provided within a vector, a cell or a liposome to said subject.

42. The method of claim 40, wherein said isolated nucleic acid has a nucleotide sequence as set forth in any one of SEQ ID NOS:23-30 or 62-63, or a sequence homologous thereto.

43. A method for enhancing the expression of insulin in a pancreatic ß-type islet cell, comprising contacting a pancreatic ß-type islet cell with an amount of a Cgen-G11 GLP-1 splice variant having an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61, or a derivative thereof, under conditions that enable insulin expression, thereby enhancing the expression of insulin in a pancreatic ß-type islet cell.

44. A method for enhancing the expression of insulin in a pancreatic ß-type islet cell, comprising contacting a pancreatic ß-type islet cell with an amount of an isolated nucleic acid encoding for a Cgen-G11 GLP-1 splice variant, wherein the Cgen-G11 GLP-1 splice variant has an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61, or a derivative thereof, under conditions that enable insulin expression, thereby enhancing the expression of insulin in a pancreatic ß-type islet cell.

45. The method of claim 44, wherein said isolated nucleic acid is provided within a vector, a cell or a liposome to said subject.

46. The method of claim 44, wherein said isolated nucleic acid has a nucleotide sequence as set forth in any one of SEQ ID NOS:23-30 or 62-63, or a sequence homologous thereto.

47. A method of reducing mortality and morbidity after myocardial infraction in a subject, comprising administering to the subject an amount of a Cgen-G11 GLP-1 splice variant having an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61, or a derivative thereof, wherein the Cgen-G11 GLP-1 splice variant is at a dosage effective to normalize blood glucose, thereby reducing mortality and morbidity after myocardial infraction in said subject.

48. A method of reducing mortality and morbidity after myocardial infraction in a subject, comprising administering to the subject an amount of an isolated nucleic acid molecule encoding for a Cgen-G11 GLP-1 splice variant, wherein said Cgen-G11 GLP-1 splice variant has an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61, or a derivative thereof, and wherein said Cgen-G11 GLP-1 splice variant is at a dosage effective to normalize blood glucose, thereby reducing mortality and morbidity after myocardial infraction in said subject.

49. The method of claim 48, wherein said isolated nucleic acid is provided within a vector, a cell or a liposome to said subject.

50. The method of claim 48, wherein said isolated nucleic acid has a nucleotide sequence as set forth in any one of SEQ ID NOS:23-30 or 62-63, or a sequence homologous thereto.

51. A method of attenuating post-surgical catabolic changes and insulin resistance, comprising administering to the subject an amount of a Cgen-G11 GLP-1 splice variant having an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61, or a derivative thereof, wherein the Cgen-G11 GLP-1 Splice Variant is insulinotropic in said subject, thereby attenuating post-surgical catabolic changes and insulin resistance.

52. A method of attenuating post-surgical catabolic changes and insulin resistance, comprising administering to the subject an amount of an isolated nucleic acid encoding for a Cgen-G11 GLP-1 splice variant, wherein the Cgen-G11 GLP-1 splice variant has an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61, or a derivative thereof, and said Cgen-G11 GLP-1 splice variant is insulinotropic in said subject, thereby attenuating post-surgical catabolic changes and insulin resistance.

53. The method of claim 52, wherein said isolated nucleic acid is provided within a vector, a cell or a liposome to said subject.

54. The method of claim 52, wherein said isolated nucleic acid has a nucleotide sequence as set forth in any one of SEQ ID NOS:23-30 or 62-63 or a sequence homologous thereto.

55. A method of attenuating post-surgical catabolic changes and hormonal responses to stress, comprising administering to the subject an amount of a Cgen-G11 GLP-1 splice variant having an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61, or a derivative thereof, wherein the Cgen-G11 GLP-1 splice variant is insulinotropic in said subject, thereby attenuating post-surgical catabolic changes and hormonal responses to stress.

56. A method of attenuating post-surgical catabolic changes and hormonal responses to stress, comprising administering to the subject an amount of an isolated nucleic acid encoding for a Cgen-G11 GLP-1 splice variant, wherein the Cgen-G11 GLP-1 splice variant has an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61, or a derivative thereof, and said Cgen-G1 1 GLP-1 splice variant is insulinotropic in said subject, thereby attenuating post-surgical catabolic changes and hormonal responses to stress.

57. The method of claim 56, wherein said isolated nucleic acid is provided within a vector, a cell or a liposome to said subject.

58. The method of claim 56, wherein said isolated nucleic acid has a nucleotide sequence as set forth in any one of SEQ ID NOS:23-30 or 62-63 or a sequence homologous thereto.

59. A method of sedating a subject, comprising administering to the subject a Cgen-G11 GLP-1 splice variant having an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61, or a derivative thereof, in an amount sufficient to produce a sedative or anti-anxiolytic effect on said subject, thereby sedating a mammalian subject.

60. A method of sedating a subject, comprising administering to the subject an isolated nucleic acid encoding for a Cgen-G11 GLP-1 splice variant, wherein the Cgen-G11 GLP-1 splice variant has an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61, or a derivative thereof, in an amount sufficient to produce a sedative or anti-anxiolytic effect on said subject, thereby sedating a mammalian subject.

61. The method of claim 60, wherein said isolated nucleic acid is provided within a vector, a cell or a liposome to said subject.

62. The method of claim 60, wherein said isolated nucleic acid has a nucleotide sequence as set forth in any one of SEQ ID NOS:23-30 or 62-63 or a sequence homologous thereto.

63. A method of reducing body weight in a subject comprising administering to the subject a Cgen-G11 GLP-1 splice variant having an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61, or a derivative thereof, in an amount sufficient to cause reduction in body weight.

64. A method of reducing body weight in a subject comprising administering to the subject an isolated nucleic acid encoding for a Cgen-G11 GLP-1 splice variant, wherein the Cgen-G11 GLP-1 splice variant has an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61, or a derivative thereof, in an amount sufficient to cause reduction in body weight.

65. The method of claim 64, wherein said isolated nucleic acid is provided within a vector, a cell or a liposome to said subject.

66. The method of claim 64, wherein said isolated nucleic acid has a nucleotide sequence as set forth in any one of SEQ ID NOS:23-30 or 62-63 or a sequence homologous thereto.

67. A method of suppressing or reducing appetite in a subject, comprising administering to the subject a Cgen-G11 GLP-1 splice variant having an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61, or a derivative thereof, in an amount sufficient to cause suppression or reduction of appetite.

68. A method of suppressing or reducing appetite in a subject, comprising administering to the subject an isolated nucleic acid encoding for a Cgen-G11 GLP-1 splice variant, wherein the Cgen-G11 GLP-1 splice variant has an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61, or a derivative thereof, in an amount sufficient to cause suppression or reduction of appetite in said subject.

69. The method of claim 68, wherein said isolated nucleic acid is provided within a vector, a cell or a liposome to said subject.

70. The method of claim 68, wherein said isolated nucleic acid has a nucleotide sequence as set forth in any one of SEQ ID NOS:23-30 or 62-63 or a sequence homologous thereto.

71. A method of increasing an insulinotropic response in ischemia injured brain cells comprising contacting ischemia injured brain cells with a Cgen-G11 GLP-1 splice variant having an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61, or a derivative thereof, wherein said Cgen-G11 GLP-1 splice variant is insulinotropic, thereby increasing an insulinotropic response in ischemia injured brain cells.

72. A method of controlling stroke-related hyperglycemia in a subject, comprising administering to the subject having suffered a stroke a Cgen-G11 GLP-1 splice variant having an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61, or a derivative thereof, wherein the Cgen-G11 GLP-1 splice variant is insulinotropic and neuroprotective in said subject, thereby controlling stroke-related hyperglycemia.

73. A method of promoting weight gain in a subject, comprising administering to the subject an inhibitor of a Cgen-G11 GLP-1 splice variant, wherein the GLP-1 splice variant has an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61 or is a derivative thereof, in an amount sufficient to cause weight gain in said subject.

74. The method of claim 73, wherein said method is utilized for the treatment of anorexia and other wasting diseases.

75. A method of promoting weight gain in a subject, comprising:

administering to the subject an isolated nucleic acid capable of hybridizing to a nucleic acid encoding for a Cgen-G11 GLP-1 Splice Variant, wherein the GLP-1 Splice Variant has an amino acid sequence as set forth in any one of SEQ ID NOS:7-22 or 58-61 or is a derivative thereof;

and wherein the nucleic acid is administered in an amount sufficient to prevent or diminish the expression of said GLP-1 Splice Variant, thereby causing weight gain in said subject.

76. The method of claim 75, wherein said isolated nucleic acid is provided within a vector, a cell or a liposome to said subject.

77. The method of claim 75, wherein said isolated nucleic acid has a nucleotide sequence as set forth in any one of SEQ ID NOS:23-30 or 62-63, a sequence homologous thereto, or an oligonucleotide fragment of at least about 12 nucleotides thereof.

78. The method of claim 75, wherein said isolated nucleic acid is selected from the group consisting of: antisense nucleotide sequence, sense nucleotide sequence, short interfering RNA, ribozyme and aptamer.

79. The method of claim 75, wherein said method is utilized for the treatment of anorexia and other wasting diseases.

80. A method for detecting Cgen-O11 OXM splice variants in a biological sample, comprising the steps of: contacting said biological sample with an antibody specifically recognizing the isolated Cgen-O11 OXM splice variant of claim 20 under conditions whereby said antibody specifically interacts with a Cgen-O11 OXM splice variant in said biological sample, and detecting said interaction; wherein the presence of said interaction correlates with the presence of a splice variant in the biological sample.

81. The method of claim 80, wherein said antibody specifically recognizes a Cgen-O11 OXM splice variant peptide fragment of claim 21.

82. A method for detecting Cgen-O11 OXM splice variant nucleic acid sequences in a biological sample, comprising the steps of: hybridizing the isolated nucleic acid molecule of claim 28, or an oligonucleotide fragment of at least about 12 nucleotides thereof to a nucleic acid material of said biological sample and detecting said hybridization complex; wherein the presence of said hybridization complex correlates with the presence of a splice variant nucleic acid sequence in the said biological sample.

83. A method for detecting Cgen-O11 OXM splice variant nucleic acid sequences in a biological sample, comprising the steps of: hybridizing an isolated nucleic acid molecule encoding for a Cgen-O11 OXM splice variant peptide fragment of claim 32, or a fragment of at least about 12 nucleotides thereof to a nucleic acid material of said biological sample and detecting said hybridization complex; wherein the presence of said hybridization complex correlates with the presence of a splice variant nucleic acid sequence in the said biological sample.

84. A method for treating maturity onset diabetes mellitus in a subject comprising administering to the subject an amount of a Cgen-O11 OXM splice variant having an amino acid sequence as set forth in any one of SEQ ID NOS:41-44, or a derivative thereof, wherein the Cgen-O11 OXM splice variant is insulinotropic in said subject, thereby treating maturity onset diabetes mellitus in said subject.

85. A method for treating maturity onset diabetes mellitus in a subject comprising administering to the subject an amount of an isolated nucleic acid encoding a Cgen-O11 OXM splice variant, wherein the Cgen-O11 OXM splice variant has an amino acid sequence as set forth in any one of SEQ ID NOS:41-44, or a derivative thereof, and said Cgen-O11 OXM splice variant is insulinotropic in said subject, thereby treating maturity onset diabetes mellitus in said subject.

86. The method of claim 85, wherein said isolated nucleic acid is provided within a vector, a cell or a liposome to said subject.

87. The method of claim 85, wherein said isolated nucleic acid has a nucleotide sequence as set forth in any one of SEQ ID NOS:45-48 or a sequence homologous thereto.

88. A method for enhancing the expression of insulin in a pancreatic ß-type islet cell, comprising contacting a pancreatic ß-type islet cell with an amount of a Cgen-O11 OXM Splice Variant having an amino acid sequence as set forth in any one of SEQ ID NOS:41-44, or a derivative thereof, under conditions that enable insulin expression, thereby enhancing the expression of insulin in a pancreatic ß-type islet cell.

89. A method for enhancing the expression of insulin in a pancreatic ß-type islet cell, comprising contacting a pancreatic ß-type islet cell with an amount of an isolated nucleic acid encoding a Cgen-O11 OXM splice variant, wherein the Cgen-O11 OXM splice variant has an amino acid sequence as set forth in any one of SEQ ID NOS:41-44, or a derivative thereof, under conditions that enable insulin expression, thereby enhancing the expression of insulin in a pancreatic ß-type islet cell.

90. The method of claim 89, wherein said isolated nucleic acid is provided within a vector, a cell or a liposome to said subject.

91. The method of claim 89, wherein said isolated nucleic acid has a nucleotide sequence as set forth in any one of SEQ ID NOS:45-48 or a sequence homologous thereto.

92. A method of reducing body weight in a subject comprising administering to the subject a Cgen-O11 OXM splice variant having an amino acid sequence as set forth in any one of SEQ ID NOS:41-44, or a derivative thereof, in an amount sufficient to cause reduction in body weight.

93. A method of reducing body weight in a subject comprising administering to the subject an isolated nucleic acid encoding a Cgen-O11 OXM splice variant, wherein the Cgen-O11 OXM splice variant has an amino acid sequence as set forth in any one of SEQ ID NOS:41-44, or a derivative thereof, in an amount sufficient to cause reduction in body weight.

94. The method of claim 93, wherein said isolated nucleic acid is provided within a vector, a cell or a liposome to said subject.

95. The method of claim 91, wherein said isolated nucleic acid has a nucleotide sequence as set forth in any one of SEQ ID NOS:45-48 or a sequence homologous thereto.

96. A method of suppressing or reducing appetite in a subject, comprising administering to the subject a Cgen-O11 OXM splice variant, having an amino acid sequence as set forth in any one of SEQ ID NOS:41-44 or a derivative thereof, in an amount sufficient to cause suppression or reduction of appetite.

97. A method of suppressing or reducing appetite in a subject, comprising administering to the subject an isolated nucleic acid encoding for a Cgen-O11 OXM splice variant, wherein the Cgen-O11 OXM splice variant has an amino acid sequence as set forth in any one of SEQ ID NOS:41-44, or a derivative thereof, in an amount sufficient to cause suppression or reduction of appetite in said subject.

98. The method of claim 97, wherein said isolated nucleic acid is provided within a vector, a cell or a liposome to said subject.

99. The method of claim 97, wherein said isolated nucleic acid has a nucleotide sequence as set forth in any one of SEQ ID NOS:45-48 or a sequence homologous thereto.

100. A method of promoting weight gain in a subject, comprising administering to the subject an inhibitor of a Cgen-O11 OXM splice variant, wherein the OXM splice variant has an amino acid sequence as set forth in any one of SEQ ID NOS:41-44 or is a derivative thereof, in an amount sufficient to cause weight gain in said subject.

101. The method of claim 100, wherein said method is utilized for the treatment of anorexia and other wasting diseases.

102. A method of promoting weight gain in a subject, comprising:

administering to the subject an isolated nucleic acid capable of hybridizing to a nucleic acid encoding for a Cgen-O11 OXM splice variant, wherein the OXM splice variant has an amino acid sequence as set forth in any one of SEQ ID NOS:41-44 or is a derivative thereof;

and wherein the nucleic acid is administered in an amount sufficient to prevent or diminish the expression of said OXM Splice Variant, thereby causing weight gain in said subject.

103. The method of claim 102, wherein said isolated nucleic acid is provided within a vector, a cell or a liposome to said subject.

104. The method of claim 102, wherein said isolated nucleic acid has a nucleotide sequence as set forth in any one of SEQ ID NOS:45-48, a sequence homologous thereto, or is an oligonucleotide fragment of at least about 12 nucleotides thereof.

105. The method of claim 102, wherein said isolated nucleic acid is selected from the group consisting of: antisense nucleotide sequence, sense nucleotide sequence, short interfering RNA, ribozyme and aptamer.

106. The method of claim 102, wherein said method is utilized for the treatment of anorexia and other wasting diseases.

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MKSIYFVAGLFVMLVQGSWQRSLQDTEEKSRSFSASQADPLSDPDQMNEDKRHSQGTFTSDYSKYLDSRR - Variant

AQDFVQWLMNTKRNRNNIAKRHDEFERHAEGTFTSDVSSYLEGQAAKEFIAWLVKGRGRRDFPEEVAIVE - WT
AQDFVQWLMNTKRNRNNIAKRHDEFERHAEGTFTSD F PRR - Variant

ELGRRHADGSFSDEMNTILDNLAARDFINWLIQTKITDRK - WT
GR HC - Variant

% Identity: 112/180 = 62 %

Figure 1

```
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gaacgaggacaagcgccattcacagggcacattcaccagtgactacagcaagtatctggactccaggcgt - Variant

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aatttgagagacatgctgaagggacctttaccagtgat                                  - Variant

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Figure 2

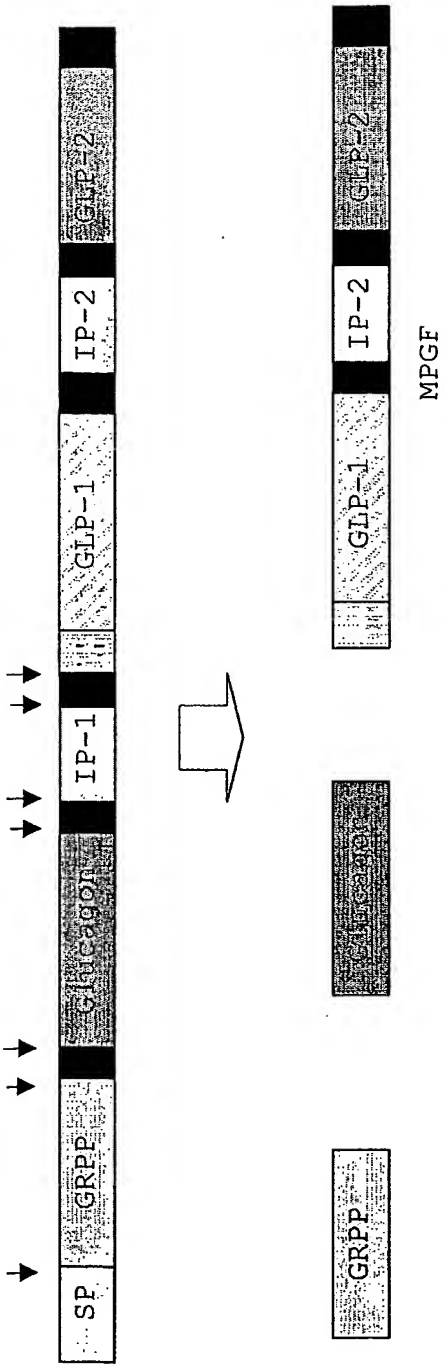
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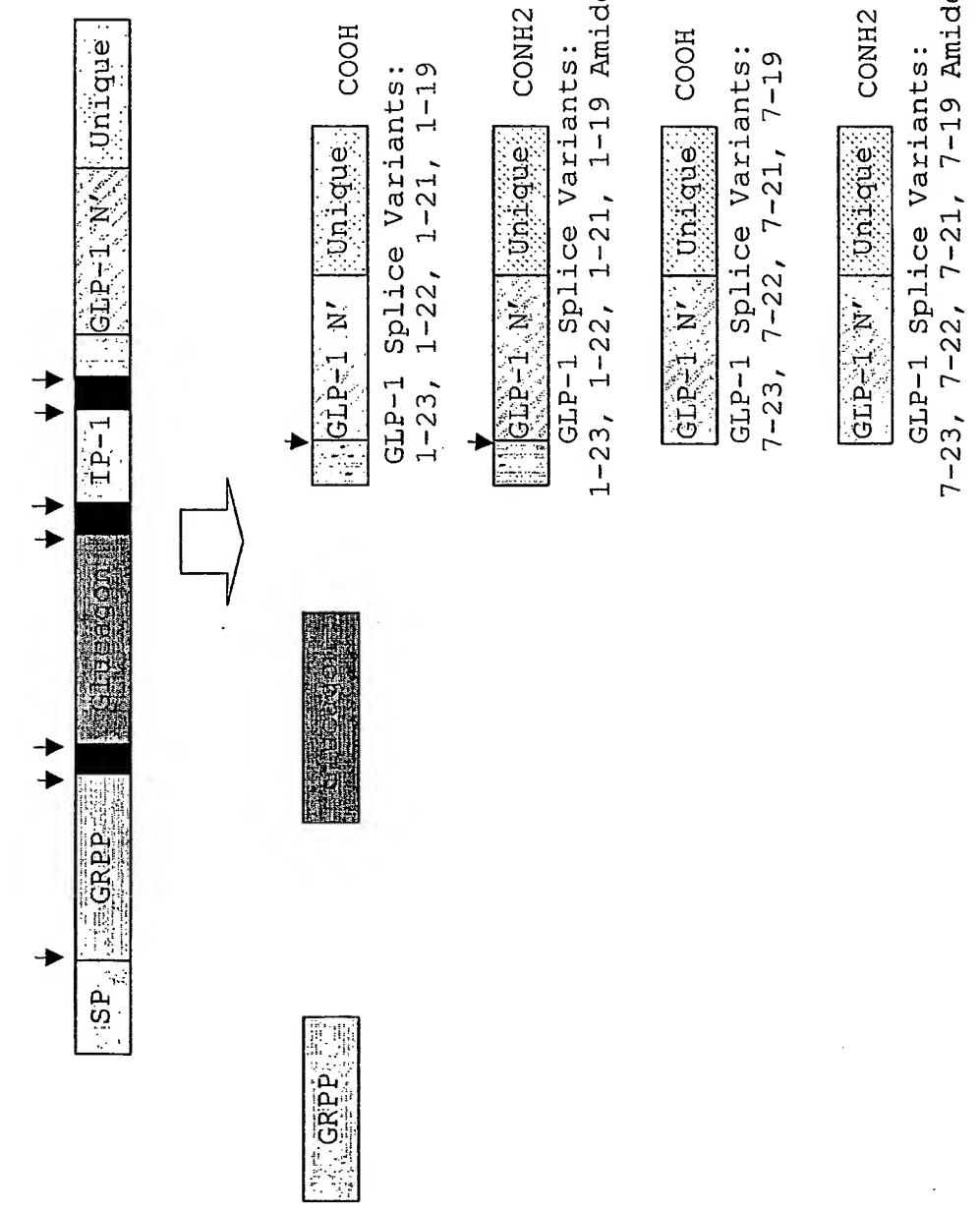
FIG. 3A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GLP-1 N'
Unique
COOH
GLP-1 Splice Variants:
7-23, 7-22, 7-21, 7-19
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7-23, 7-22, 7-21, 7-19 Amide

FIG. 3B

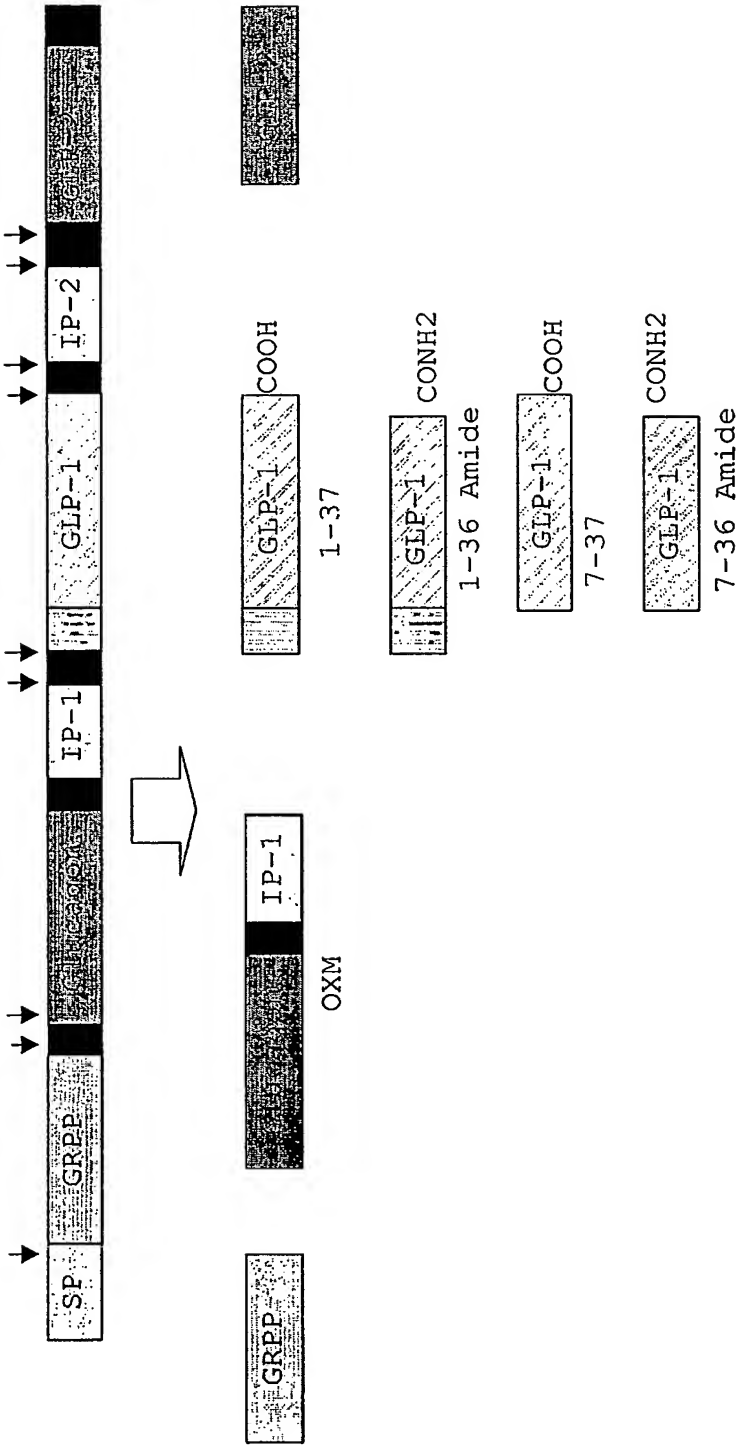
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FIG. 3C

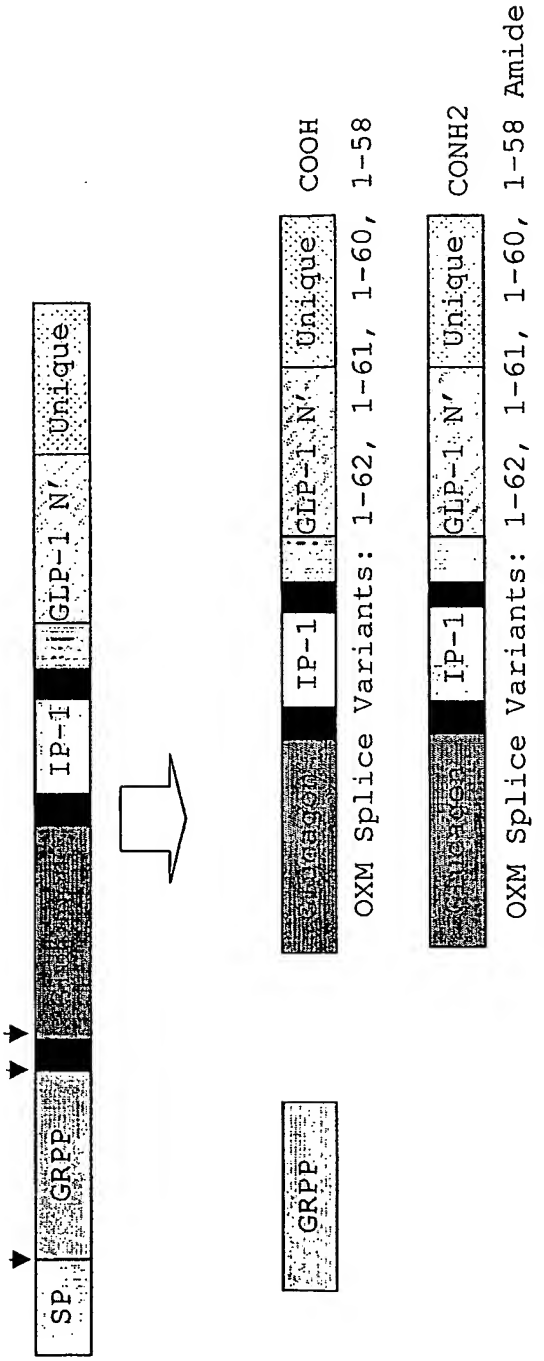
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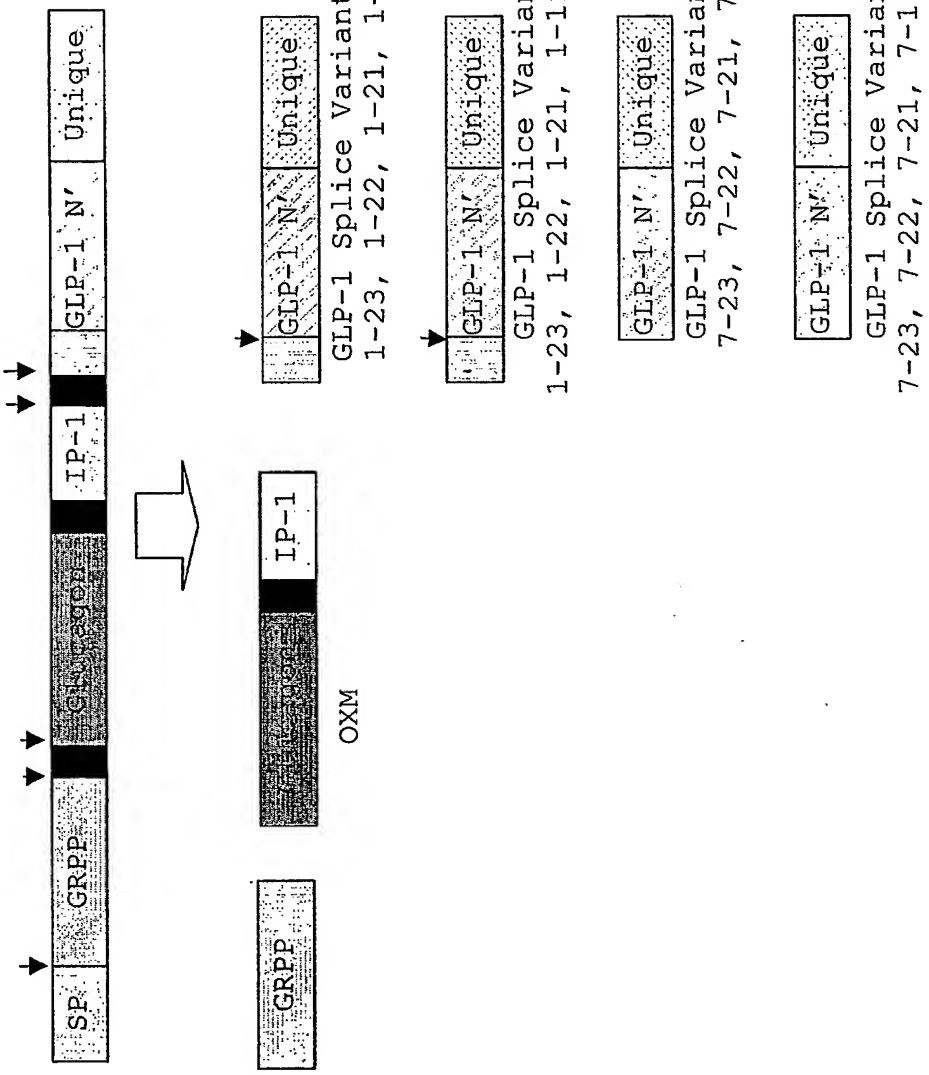
FIG. 3D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GLP-1 N'
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CONH2
GLP-1 Splice Variants:
1-23, 1-22, 1-21, 1-19 Amide
GLP-1 N'
Unique
COOH
GLP-1 Splice Variants:
7-23, 7-22, 7-21, 7-19
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Unique
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GLP-1 Splice Variants:
7-23, 7-22, 7-21, 7-19 Amide

FIG. 3E

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OXM Splice Variants:

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FIG. 4A

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GLP-1 Splice Variants:

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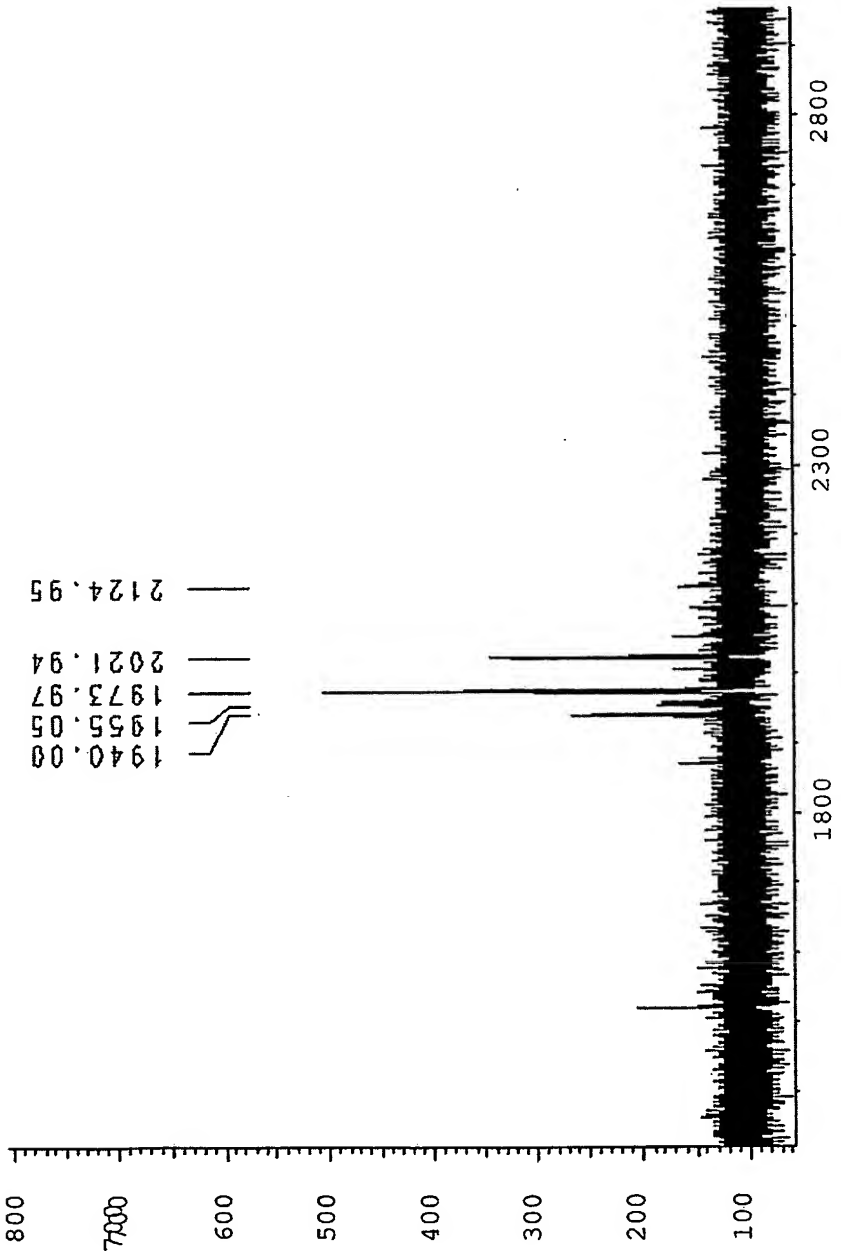
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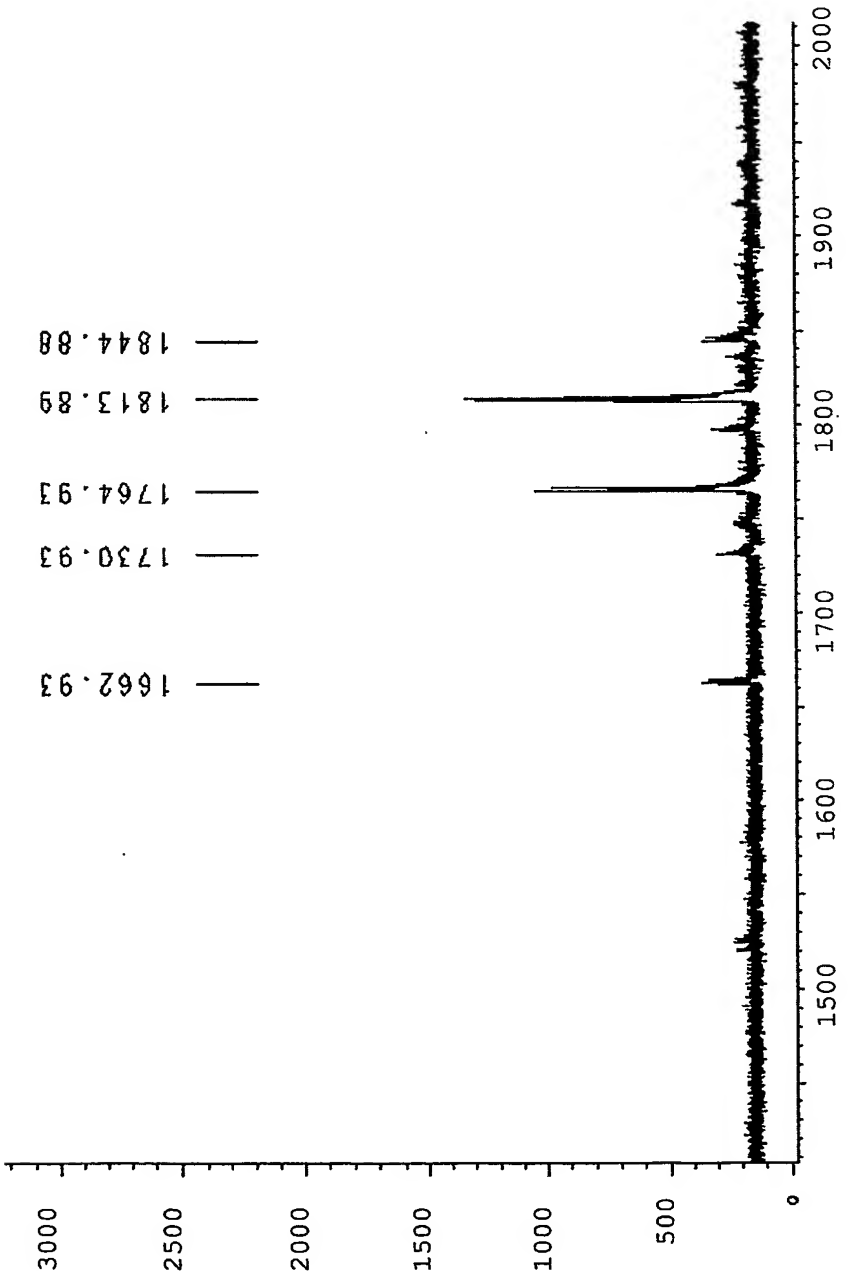
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Figure 5B

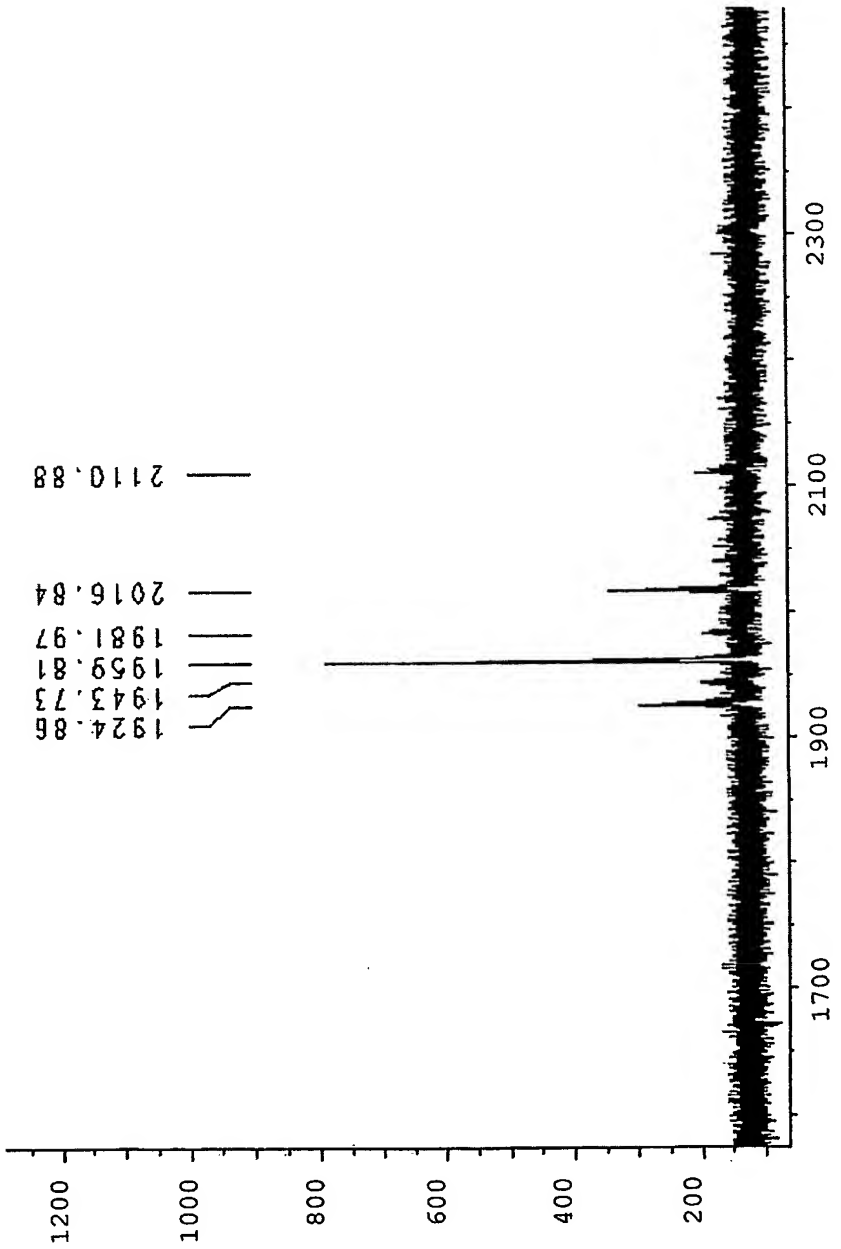
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Figure 5C

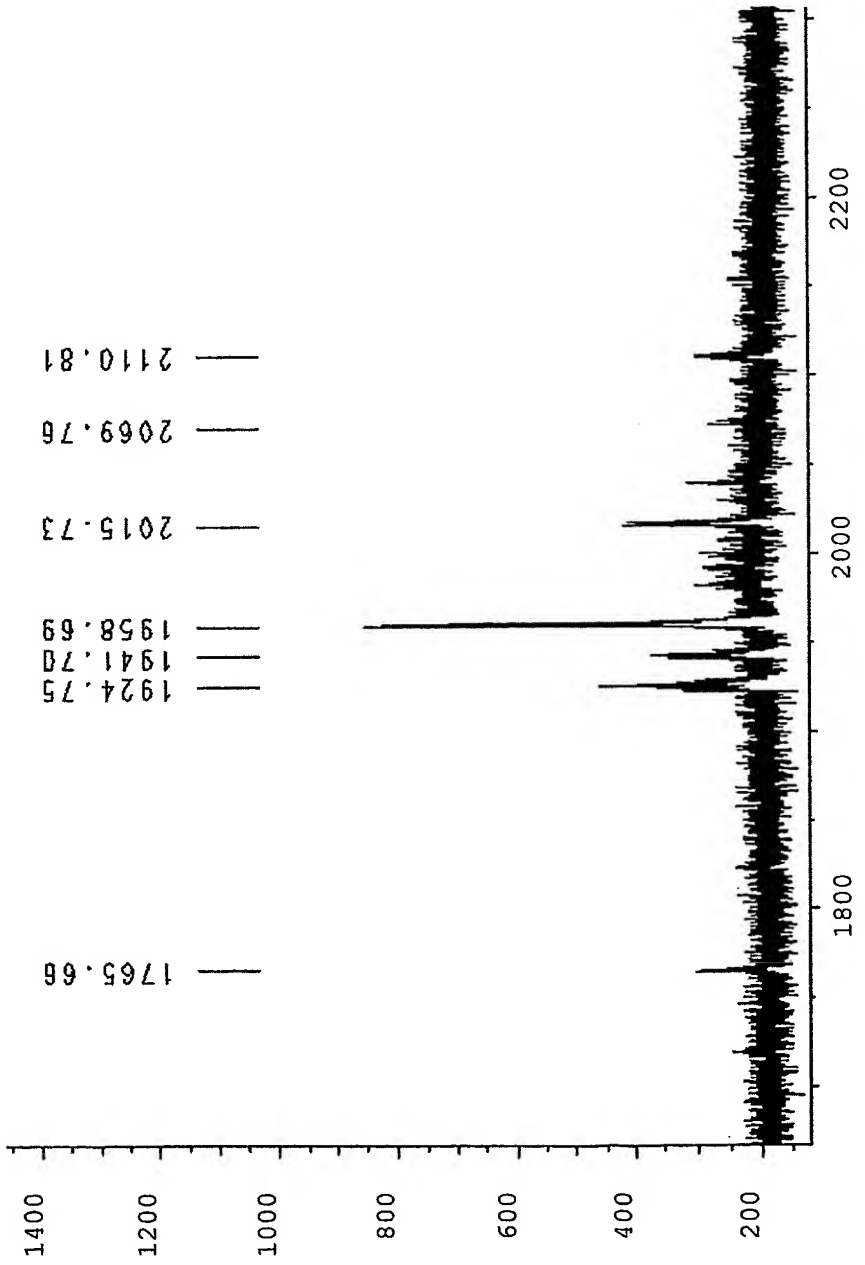
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Figure 5D

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20 25 30

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Cys Xaa

INTERNATIONAL SEARCH REPORT

Intern; al Application No
PCT/IL2004/000952

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C07K14/605 A61K38/26 C07K16/18 C12Q1/68
G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, Sequence Search, BIOSIS, PASCAL, CHEM ABS Data, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01/36632 A (AZAR IDIT ; KHOSRAVI RAMI (IL); COMPUGEN LTD (IL); DAVID ANAT (IL); LE) 25 May 2001 (2001-05-25) cited in the application page 38, line 35 - page 39, line 2; claims 1-58; figure 7; sequences 7,94	1-38, 80-83
X	DATABASE EMBL 9 February 1996 (1996-02-09), TAKEDA,J.: "EST similar to glucagon." XP002312100 retrieved from EBI Database accession no. D82172 cited in the application the whole document	1-20

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 December 2004	10/01/2005

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Gurdjian, D

Form PCT/ISA/210 (second sheet) (January 2004)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IL2004/000952

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL EST(expressed sequence tag). 9 February 1996 (1996-02-09), TAKEDA,J.: XP002312101 retrieved from EBI Database accession no. D82276 cited in the application the whole document	1-20
X	IRWIN D M ET AL: "Trout and chicken proglucagon: alternative splicing generates mRNA transcripts encoding glucagon-like peptide 2." MOLECULAR ENDOCRINOLOGY (BALTIMORE, MD.) MAR 1995, vol. 9, no. 3, March 1995 (1995-03), pages 267-277, XP002312098 ISSN: 0888-8809 abstract; figures 1,4-6	1-38
X	YEUNG C M ET AL: "Identification of a proglucagon cDNA from Rana tigrina rugulosa that encodes two GLP-1s and that is alternatively spliced in a tissue-specific manner." GENERAL AND COMPARATIVE ENDOCRINOLOGY. NOV 2001, vol. 124, no. 2, November 2001 (2001-11), pages 144-151, XP002312099 ISSN: 0016-6480 abstract; figures 1,2	1-38
X	WO 03/022304 A (GHATEI MOHAMMAD ALI ; IMP COLLEGE INNOVATIONS LTD (GB); SMALL CAROLINE) 20 March 2003 (2003-03-20) page 16, line 26 - page 17, line 9 page 2, line 24 - page 3, line 22 abstract	1-38, 80-83
X	EP 0 795 562 A (KYOWA HAKKO KOGYO KK) 17 September 1997 (1997-09-17) the whole document	1-38, 80-83
A	DRUCKER D J: "GLUCAGON AND THE GLUCAGON-LIKE PEPTIDES" PANCREAS, RAVEN PRESS, NEW YORK, NY, US, vol. 5, no. 4, 1990, pages 484-488, XP000650242 ISSN: 0885-3177 the whole document	1-38, 80-83

INTERNATIONAL SEARCH REPORT

Intl tional application No.
PCT/IL2004/000952

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. type of material

   [X] a sequence listing

   [ ] table(s) related to the sequence listing

   b. format of material

   [X] in written format

   [X] in computer readable form

   c. time of filing/furnishing

   [X] contained in the international application as filed

   [ ] filed together with the international application in computer readable form

   [ ] furnished subsequently to this Authority for the purpose of search

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL2004/000952

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 39-79,84-102 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [X] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern al Application No
PCT/IL2004/000952

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 0136632	A	25-05-2001	AU	1410801 A	30-05-2001
			WO	0136632 A2	25-05-2001
WO 03022304	A	20-03-2003	BR	0212374 A	17-08-2004
			CA	2459862 A1	20-03-2003
			EP	1427437 A1	16-06-2004
			WO	03022304 A1	20-03-2003
EP 0795562	A	17-09-1997	CA	2204645 A1	17-05-1996
			EP	0795562 A1	17-09-1997
			US	5858975 A	12-01-1999
			WO	9614336 A1	17-05-1996